

Differences in Short-Term Cardiovascular Responses to Active Standing Orthostatic Stress

Test between Individuals with and without Long-COVID

by

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General Abstract

Long-COVID, attributed to infection by the severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2), affects approximately 400 million individuals globally. The condition, clinically characterized as a multi-system condition, with persistent, worsening, or fluctuating symptoms, lasting at least three months after the acute phase and not explainable by alternative diagnosis, is profoundly debilitating and associated with all-cause morbidity. While emerging research identifies the prevalence of associated symptoms, a significant gap exists in characterizing patterns of physiological responses across body systems, especially during physiological stress. This thesis is structured in two parts: (1) a systematic review and (2) an observational cross-sectional study. The systematic review aims to synthesize existing literature on physiological system responses (e.g. cardiovascular, respiratory, autonomic) to an active standing orthostatic stress test, and the cross-sectional study addresses identified gaps by examining cardiovascular response patterns in individuals with long-COVID compared to individuals without long-COVID. Investigating and analyzing these responses over time will contribute to a comprehensive understanding of the underlying mechanisms of dysfunction such as orthostatic hypotension, often observed and reported in individuals with long-COVID. It would also aid in our understanding of compensatory dynamics during an active standing orthostatic stress test in the long-COVID population.

Contributions of Authors

This thesis follows a sandwich-style manuscript format, with content in chapters 4 and 5 prepared as standalone manuscripts for publication. Chapter 4 presents a systematic review titled “*Using active standing orthostatic stress test to assess physiological responses in individuals with long-COVID: A systematic review*”, which is intended for journal submission. Chapter 5 is a cross-sectional study carried out originally as proposed during my master’s thesis proposal, with some modifications made due to time constraints and is titled “*Unveiling the unseen: Exploring orthostatic hypotension and intolerance symptoms in long-COVID*”. Specific author’s contributions to chapters 4 and 5 are outlined below.

Chapter 4: Faith Olarinde: Concept development, data collection, data analysis, manuscript writing, review & editing. **Albena Nunes-Silva:** Data collection and analysis. **Diana Sanchez-Ramirez & Yannick Molgat-Seon:** Manuscript feedback and revision. **Rodrigo Villar:** Concept development, project supervision and advising, manuscript feedback and revisions.

Chapter 5: Faith Olarinde: Concept development, data collection, data analysis, manuscript writing, review & editing. **Dihogo de Matos & Jefferson Lima de Santana:** Methodology conceptualization and manuscript feedback. **Diana Sanchez-Ramirez & Yannick Molgat-Seon:** Manuscript feedback and revision. **Rodrigo Villar:** Concept development, project supervision and advising, manuscript feedback and revisions.

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In 2022, I came across a quote that said university isn't meant to train you for one job, but to give you a "Swiss Army knife" brain, to expose you to different ways of thinking and help you learn how to approach and solve problems. This was the mindset through my masters', and I know I wouldn't have developed it fully without the support of the amazing people in my life.

To Dr. Villar: thank you for your support and encouragement throughout my master's and undergrad. Your advice, belief in me, and kindness have left a lasting impact, and I'll always carry your words with me. I deeply admire the way you value people for who they are and hope to emulate that in my own journey.

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Dedication

This thesis is dedicated to my late father, Olushola Michael. Thank you for teaching me the value of education and the importance of sharing knowledge with others. Your influence lives on in everything I do.

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List of abbreviations

AST	Active standing test
BP	Blood pressure
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CO	Cardiac output
CO-SVR	Cardiac output – Systemic vascular resistance
COVID-19	Coronavirus disease 2019
CSEP	Canadian Society for Exercise Physiology
DBP	Diastolic blood pressure
DSQ-SF	DePaul Symptom Questionnaire-Short form
ECG	Electrocardiogram
FDR	False discovery rate
HR	Heart rate
HUTT	Head-up tilt test
IQR	Interquartile range
LC	Long-COVID
MAP	Mean arterial pressure
ME/CFS	Myalgia encephalomyelitis/chronic fatigue syndrome
NASEM	National Academies of Sciences, Engineering and Medicine
NIBP	Non-invasive blood pressure
NIH	National Institutes of Health
NLC	Non-long-COVID

OH	Orthostatic hypotension
OI	Orthostatic intolerance
PASC	Post-acute sequelae of SARS-CoV-2
PCR	Polymerase chain reaction
PICO	Population, Intervention, Comparison, Outcome
PRISMA	Preferred reporting items for systematic reviews and meta-analysis
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBP	Systolic blood pressure
SV	Stroke volume
SVR	Systemic vascular resistance
WHO	World Health Organization

CHAPTER 1: GENERAL INTRODUCTION

1.1 A global pandemic

When a novel coronavirus was first identified in December 2019, the magnitude of its subsequent global impact was beyond human imagination. By March 2020, the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease 2019 (commonly referred to as COVID-19), was declared a pandemic by the World Health Organization (WHO) (Cucinotta & Vanelli, 2020; Zhu et al., 2020). This led to a global lockdown period lasting about two years, during which approximately 770 million individuals were infected, and 6.9 million deaths were reported worldwide (World Health Organization, 2025).

Although many infected individuals recovered to their pre-infection health state, reports of persistent dysfunction in various physiological systems – cardiovascular, autonomic, respiratory, cerebrovascular, gastrointestinal, etc., continues to impact individuals long after the infection's acute phase (initial 4 weeks) is passed (Evcik, 2023; Ferreira et al., 2024; Novak et al., 2022).

1.2 The emergence of long-COVID

Long-COVID, also referred to as post-acute sequelae of SARS-CoV-2 infection (PASC), post-COVID-19 syndrome or chronic COVID syndrome, is clinically characterized as a multi-system condition with persistent, worsening, or new symptoms following a positive COVID-19 polymerase chain reaction (PCR) or rapid kit test (Soriano et al., 2022). While different organizational definitions exist (WHO, NIH, CDC etc.), a 2024 consensus report by the National Academies of Sciences, Engineering and Medicine (NASEM) defines it as an infection-associated chronic condition that occurs after SARS-CoV-2 infection and is present for at least 3

months as a continuous, relapsing and remitting, or progressive disease that affects one or more systems (National Academies of Sciences et al., 2024)..

The occurrence of persistent symptoms such as unexplained fatigue, shortness of breath, dizziness upon standing, cognitive impairment (“brain fog”) etc., was initially observed in severe hospitalized cases (Al-Aly et al., 2024; Davis et al., 2023a). However, while the prevalence of these symptoms was reported in at least 50-70% of hospitalized patients, 10-30% of non-hospitalized and 10-12% of vaccinated individuals also reported similar symptoms, suggesting that the occurrence is not limited to the severity of the acute illness (Al-Aly et al., 2022; Ceban et al., 2022; Davis et al., 2023). Other symptoms such as arrhythmia, pulmonary inflammation, gastrointestinal symptoms, myocardial dysfunction, etc., have also been reported as sequelae of COVID-19 in severe and mild cases (Ayres, 2020; Chang et al., 2020; Davis et al., 2023; Linschoten et al., 2020). However, other researchers note that some of these symptoms are not consistently observed and caution that symptoms as fatigue and muscle weakness could have been exacerbated by prolonged hospitalization and bed rest (Alzahrani, 2021; Bliddal et al., 2021).

A significant impact on an individual’s quality of life is therefore expected, as symptoms of cognitive dysfunction, lower physical capacity, and extreme fatigue can substantially affect the functioning and performance of daily activities (Carlile et al., 2024; O’Mahoney et al., 2023; Sanchez-Ramirez et al., 2021). A systematic review found that approximately 52% of 5232 participants reported a decrease in health-related quality of life, ~36% reported a decrease in functional capacity, ~38% reported fatigue, and ~32% reported persistent dyspnea 3 to 6 months after COVID-19 infection, highlighting the profound impact on the quality of life in these individuals (Sanchez-Ramirez et al., 2021).

Concerns have been raised about the impact of lingering effects as studies indicate that long-COVID symptoms manifest across age groups, irrespective of disease severity (asymptomatic, mild, moderate or severe) (Basaca et al., 2025; Bull-Otterson et al., 2022; Kokolevich et al., 2022). Consequently, some individuals may experience long-COVID symptoms without realizing they are related to a SARS-CoV-2 infection, especially since these symptoms overlap with those of many other health conditions (Hernandez-Romieu et al., 2022; Novak et al., 2022). The long-term prognosis of this condition also remains unclear.

Although several studies have investigated long-COVID using biomarkers to elucidate cardiovascular abnormalities (Adu-Amankwaah, 2024; Lai et al., 2023; Wu et al., 2024), there remains a substantial gap in understanding how the cardiovascular system responds dynamically to functional stressors like the active standing orthostatic stress test in the long-COVID population. This represents a significant gap, as orthostatic challenges can be used to identify subtle, yet clinically relevant dysfunctions that may contribute to common symptoms such as dizziness, light-headedness, and fatigue.

1.3 Research aims

This thesis aims to i) synthesize existing literature on the use of the active standing orthostatic stress test (AST) to assess physiological system responses (e.g. cardiovascular, respiratory, autonomic) in individuals with long-COVID, and ii) report the frequency of occurrence of orthostatic hypotension (OH) and orthostatic intolerance (OI) symptoms in individuals with long-COVID (LC); and iii) examine discrete phases of cardiovascular response patterns during an active standing orthostatic stress test. The overarching goal is to contribute to the understanding of dynamic physiologic mechanisms that may be altered in the long-COVID

population, using a phase-specific analysis to capture real-time physiological responses to postural changes.

1.4 Research questions

Systematic review: 1) What is the current state of evidence on the use of active standing orthostatic stress test to assess cardiovascular, autonomic, and respiratory responses in individuals with long-COVID? 2) What methodological limitations or gaps exist in this emerging body of literature?

Cross-sectional study: Do individuals with long-COVID show a higher frequency of occurrence of orthostatic hypotension and orthostatic intolerance symptoms and altered cardiovascular responses across specific time phases of an active standing test when compared to a group without long-COVID?

1.5 Research objectives

The systematic review chapter aims:

- 1) To identify and summarize studies published between 2020 and 2025 that use the active standing orthostatic stress test to assess physiological responses in adults with long-COVID.
- 2) To evaluate the types of physiological variables measured across studies, particularly focusing on cardiovascular, autonomic, and respiratory outcomes.
- 3) To assess methodological consistency of existing studies and explore how differences in active standing protocols may contribute to variability in findings and interpretation.

The cross-sectional study chapter aims:

- 1) To assess and compare symptom profiles using the DePaul Symptom Questionnaire – Short Form (DSQ-SF) between individuals with and without LC.

- 2) To report the frequency of individuals meeting the criteria for orthostatic hypotension (OH) and orthostatic intolerance (OI) symptoms.
- 3) To compare cardiovascular responses, specifically systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), systematic vascular resistance (SVR), heart rate (HR), cardiac output (CO), stroke volume (SV) and CO-SVR matching across specific time phases of an active standing orthostatic stress test between individuals with long-COVID (LC) and without LC with OH (NLC_{OH}) and without LC with normal blood pressure response (NLC_{NBPR}), focusing on identifying delayed or impaired compensatory responses.

1.6 Research hypothesis

The cross-sectional study hypotheses are:

- 1 **Objective 1 hypothesis:** The DSQ-SF item composite score (severity and frequency of symptoms) for the LC group would be significantly different compared to the NLC groups.
- 2 **Objective 2 hypothesis:** A higher frequency of occurrence of OH and OI symptoms will be observed in the long-COVID (LC) group compared to the NLC group.
- 3 **Objective 3 hypothesis:** A more pronounced drop in SBP and DBP, a blunted increase in HR, reduced SVR, CO, and SV, and a delayed CO-SVR matching will be observed in the LC group compared to the NLC_{OH} and NLC_{NBPR} groups.

1.7 Overall structure of thesis document

This thesis is organized into six chapters, where I used a sandwich-style document approach as outlined by the Faculty of Graduate Studies (FGS) at the University of Manitoba. The purpose is to provide a comprehensive exploration of short-term cardiovascular responses in individuals with long-COVID. It primarily consists of a systematic review and an observational cross-sectional study, which will be presented as separate but complementary chapters. The systematic review provides a synthesis of existing literature on autonomic, cardiovascular and respiratory responses to active standing orthostatic stress in the long-COVID population, aiming to identify trends and gaps. The observational cross-sectional study then aims to address some of those gaps. This two-part structure allows for not only building on existing knowledge but also aims to contribute new evidence to the field of long-COVID. Below is described the overall structure of each chapter.

Chapter 1 introduces long-COVID and outlines the research aims, questions, objectives and hypotheses.

Chapter 2 presents a review of the existing literature in the field, focusing on the cardiovascular, autonomic and respiratory effects of long-COVID. This review aims to present current key findings and identify gaps that this thesis aimed to address.

Chapter 3 presents a general methodological design details of both the systematic review and cross-sectional study.

Chapters 4 and 5 present the systematic review and the cross-sectional study in manuscript formats, respectively.

Chapter 6 presents a general discussion synthesizing the findings of the entire thesis and how it contributes to knowledge in this research area.

CHAPTER 2: LITERATURE REVIEW

This literature review covers existing research on the cardiovascular, autonomic and respiratory effects of long-COVID, with a focus on orthostatic stress testing as a tool for identifying reported cardio-autonomic dysregulation. The review identifies key findings, gaps in the literature, and the potential implications of these gaps for clinical management.

2.1 History and Pathophysiology of Coronaviruses

While often thought to be a modern virus, the history of the coronavirus dates back to the late 1930s and was first reported by Beaudette & Hudson (Hasöksüz et al., 2020; Vabret et al., 2009). Six different strains of the coronavirus namely, Human Coronavirus-229E (HCoV-229E), HCoV-OC43, HCoV-NL63, HKU1, Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) and SARS-CoV, are prevalent in humans and other mammals, with the common cold being the most common illness caused by one of the strains (Hasöksüz et al., 2020). While the first four strains – HCoV-229E, HCoV-OC43, HCoV-NL63, and HKU1 – cause mild upper respiratory disease, the MERS-CoV and SARS-CoV-2 strains have been associated with severe lower respiratory tract disease in infants, young children, and older adults (Hasöksüz et al., 2020; Vabret et al., 2009).

The first iteration of the SARS-CoV virus was identified in 2003, with a fatality rate of 9.3% (Drosten et al., 2003). Clinical symptoms, similar to the SARS-CoV-2 virus, included fever, headache, dry cough, dyspnea, and hypoxemia, subsequently leading to respiratory failure caused by alveolar damage (Davis et al., 2023; Drosten et al., 2003). Similarly, the MERS-CoV virus was declared an epidemic in 2012. Although its fatality rate was higher (34%) compared to SARS-CoV, its spread remains restricted to the Middle East, Europe, and South Asia regions (Kanwar et al., 2017; Osuchowski et al., 2021).

SARS-CoV-2 enters the human body primarily through the respiratory tract and initially presents as a respiratory disease due to the abundance of Angiotensin-Converting Enzyme 2 (ACE-2) receptors in the lungs (Mani & Griffin, 2024; Osuchowski et al., 2021). The virus's structure includes a spike protein (S protein) that has a segment that attaches to the extracellular part of the ACE-2 receptor. By binding to this receptor, the virus enters the cell, and this interaction leads to the blockage and downregulation of the ACE-2 receptor (Osuchowski et al., 2021). Figure 2.1 shows the mode of infection of the SARS-CoV-2.

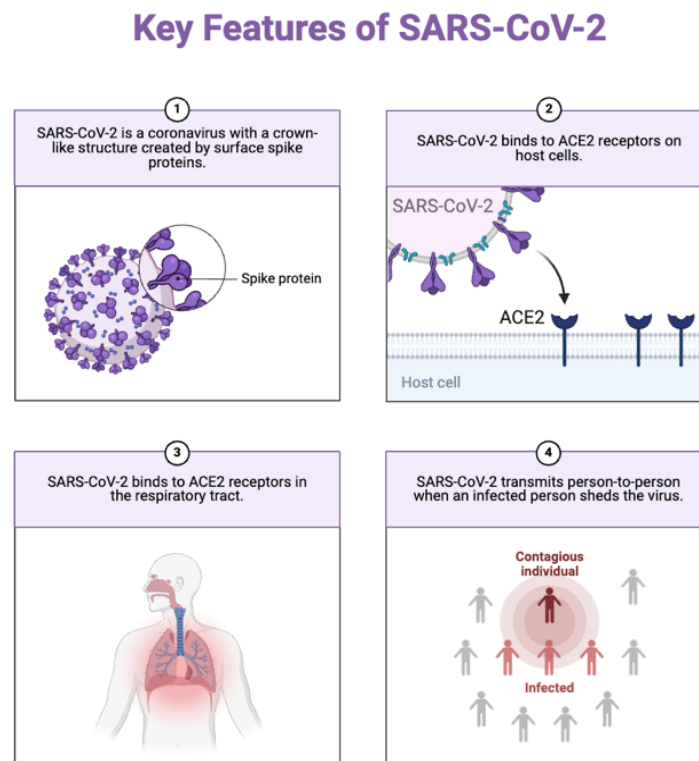


Figure 2.1: Mode of infection of SARS-CoV-2 (adapted from Biorender.com)

The presence of the ACE-2 receptors in more than 72 human tissues also allows the virus to potentially spread to other organs, such as the heart, brain, vasculature, and other organ tissues (Novak et al., 2022; Soriano et al., 2022; Zhu et al., 2020). This spread occurs through viral-cell fusion, supported by transmembrane serine protease 2 (TMPRSS2), or via endocytosis through

endosomal cysteine protease cathepsin L (CTSL) activation (Ashraf et al., 2021; Kwan et al., 2021). The virus's single-stranded ribonucleic acid (RNA) genome also carries the genetic information necessary for rapid replication and protein production (Zhu et al., 2020).

However, due to RNA replication being more prone to error compared to DNA replication, it leads to the emergence of numerous variants (Batah & Fabro, 2021). The increased susceptibility, combined with the virus's RNA nature, allows it to bind to multiple organs and mutate, making it challenging for researchers and clinicians to comprehend and keep track of the various mechanisms and potential outcomes of infection with a specific strain. Figure 2.2 represents potential sites of SARS-CoV-2 infections.

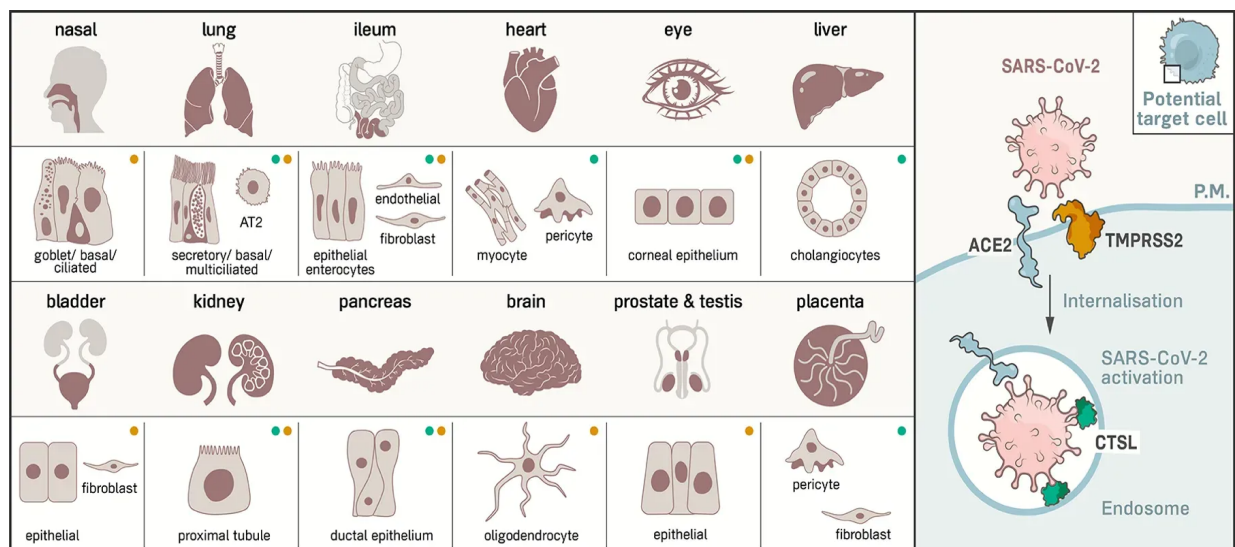


Figure 2.2: Potential sites of SARS-CoV-2 infections. Orange dots: ACE2 and TMPRSS2 are present. Green dots: ACE2 and CTSL are present (Adapted from Baraniuk, 2020).

The time from exposure to the virus to the manifestation of symptoms, known as the incubation period, is approximately 5.5 days but can range from 2 to 12 days, with some outliers (Mani & Griffin, 2024). The disease progression is typically divided into three phases: the early infection phase, which resolves on its own in about 80% of patients; the pulmonary phase, which

20% of patients will experience; and the hyperinflammatory phase, which occurs in about 6% of patients (Diab et al., 2021). The peak viral load, which is typically when the onset of symptoms begins, occurs on average by day 4, with the most infectious period ranging from days 4 to 8. Typically, the pulmonary phase begins after the 9th day, with patients experiencing shortness of breath around day 10, and some progressing severely enough to require hospital admission by day 12. The hyperinflammatory phase is marked by the onset of sepsis between days 14 and 15, acute respiratory distress syndrome by day 16, and acute cardiac and kidney injury by day 20, with death occurring around day 24 for non-survivors. However, survivors are typically discharged from the hospital around day 27 (Diab et al., 2021). Figure 2.3 represents the proposed average timeline of COVID-19 disease progression in days.

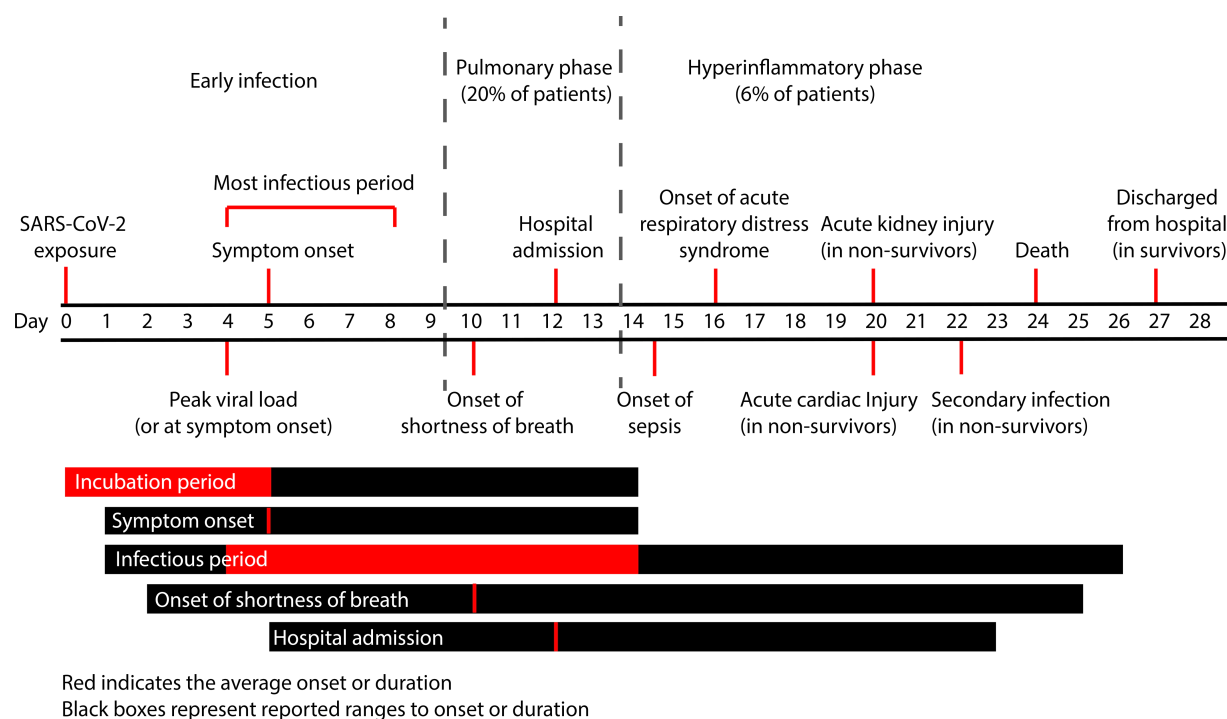


Figure 2.3: Timeline of COVID-19 disease progression in days (adapted from Diab et al., 2021).

2.2 Long-COVID: Definitions and a Consensus Report

The diagnosis of COVID-19 through virologic testing has been a cornerstone method since the disease's first identification. This diagnostic approach consists of detection through the presence of the virus using either an antigen test kit or nucleic acid amplification test, such as PCR, which has been widely accepted and used for confirming COVID-19 infections (Bliddal et al., 2021). The World Health Organization (WHO) and the National Institutes of Health also developed standardized categories to classify the severity of COVID-19 infections (see Appendix A). These categories range from asymptomatic cases, where individuals show no symptoms despite being infected, to critical cases, where individuals are hospitalized in intensive care units. This categorization provides clinicians and researchers with a way to assess patients' clinical status. Figure 2.4 presents the diagnosis of long-COVID based on Srikanth et al. 2023.

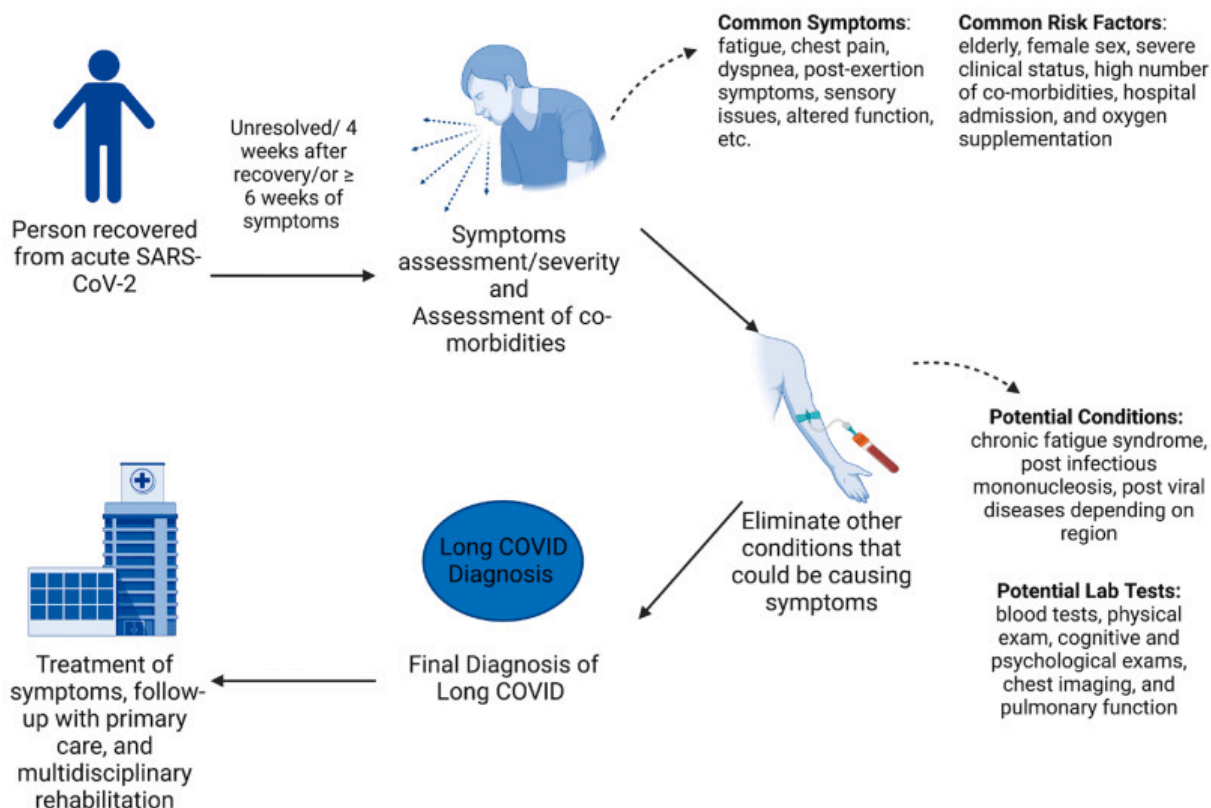


Figure 2.4: Diagnosis of long-COVID (adapted from Srikanth et al. 2023).

Asymptomatic cases present as individuals with no noticeable symptoms; however, these individuals can still spread the virus to others at a rate of one infected person to 1.4 to 4 new individuals (Liu et al., 2020). Mild cases typically exhibit symptoms such as fever, cough, fatigue, and loss of taste and smell, but do not require hospitalization. Moderate cases, however, start to present with more pronounced symptoms such as shortness of breath and are recommended to be closely medically monitored. A hallmark of moderate cases that separates them from more severe cases is an arterial oxygen saturation (SaO_2) of $\geq 94\%$ on room air at sea level. Severe cases involve more significant respiratory distress, marked by a SaO_2 of less than 94% on room air at sea level, with symptoms of severe pneumonia, difficulty breathing, and persistent cough. These cases require hospitalization and advanced medical interventions such as mechanical ventilation to assist with breathing. The final classification group is critical cases, which are life-threatening and require intensive care unit (ICU) admission due to complications such as respiratory failure, septic shock, and multi-organ dysfunction (COVID-19 Treatment Guidelines Panel, 2021.; World Health Organization, 2021). Figure 2.5 displays the classification of COVID-19 severity according to the WHO.

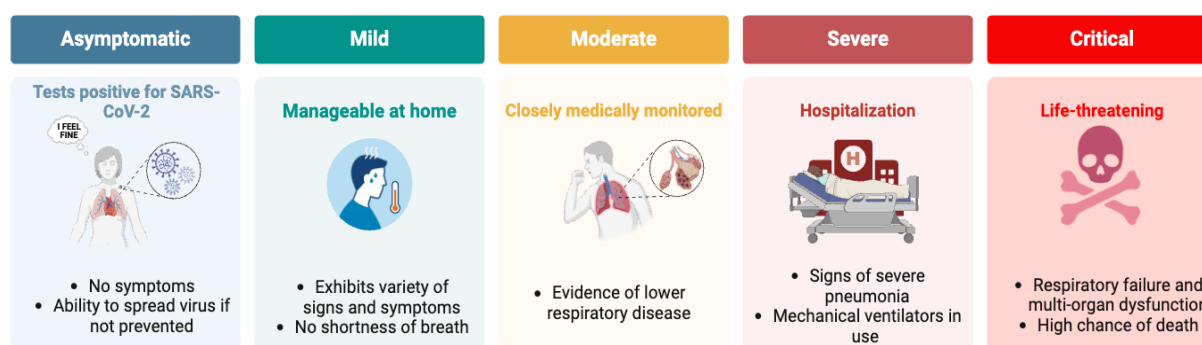


Figure 2.5: Classification of COVID-19 severity according to WHO (created in Biorender.com).

The definition of long-COVID, a condition that persists after the acute phase of the COVID-19 infection, has undergone numerous iterations and has several working definitions

(National Academies of Sciences et al., 2024). This lack of a consensus definition has posed challenges for clinicians, researchers, patients, and healthcare providers, making it difficult to have a universally accepted understanding of the condition. Although the term “long-COVID” is most commonly used to describe the lingering symptoms associated with the infection, various major health organizations employ a range of related terms to refer to this condition.

The World Health Organization (WHO) refers to it as the “post COVID-19 condition (PCC),” describing it as the ongoing or developing of new symptoms 3 months after the initial SARS-CoV-2 infection, with the symptoms lasting for at least 2 months with no alternative explanation (Soriano et al., 2022). The Centers for Disease Control and Prevention (CDC), on the other hand, uses the terms “post-acute sequelae of SARS-CoV-2 infection (PASC),” “long-COVID” and “Post-COVID Conditions (PCC),” and emphasizes the infection as a chronic condition that affects one or more organ systems with continuous, relapsing, and remitting, or progressive symptoms that lasts for at least 3 months (Centers for Disease Control and Prevention, 2024). The National Institutes of Health (NIH) uses the terms “long-COVID”, “long-haul COVID”, “PCC”, “chronic COVID” and “PASC”, and defines the condition as health problems that people experience a few months after a COVID-19 diagnosis (National Institutes of Health, 2024).

The variation in terminology and definitions reflects the diverse range of symptoms and uncertainty surrounding the precise physiological mechanisms that underlie the condition. This ambiguity in definitions also significantly implicates diagnoses, treatments, and even research on the condition (National Academies of Sciences et al., 2024). Therefore, in June of 2024, the National Academies of Sciences, Engineering and Medicine Standing Committee on Emerging Infectious Diseases and 21st Century Health Threats (NASEM) released a proposed consensus

report regarding the definition of long-COVID, which they defined using the term “long-COVID” as an “infection-associated chronic condition (IASS) that occurs after SAR-CoV-2 infection and is present for at least 3 months as a continuous, relapsing and remitting, or progressive disease state that affects one or more organ systems (National Academies of Sciences et al., 2024). Figure 2.6 displays the definitions of long-COVID used by several well-known agencies (WHO, CDC, NIH, OASH, NICE).

 World Health Organization	Post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Symptoms might also fluctuate or relapse over time (Soriano et al., 2022).
 Centers for Disease Control and Prevention	*Long- COVID is a wide range of new, returning, or ongoing health problems that people experience after being infected with the virus that causes COVID-19. Most people with COVID-19 get better within a few days to a few weeks after infection, so at least 4 weeks after infection is the start of when Long COVID could first be identified (CDC, 2024).
 National Institutes of Health Turning Discovery Into Health	*Recovery from infection with SARS-CoV-2, the virus that causes COVID-19, can vary from person to person. Most patients seem to recover quickly and completely, while others report symptoms that persist for weeks or even months after the acute phase of illness has passed (a condition often referred to as “Long COVID”). Collectively, these long-term effects of the virus are called post-acute sequelae (PASC) (NIH, 2024)
 Office of the Assistant Secretary for Health LEADING AMERICA TO HEALTHIER LIVES	Long COVID is broadly defined as signs, symptoms, and conditions that continue or develop after initial COVID-19 or SARS-CoV-2 infection. The signs, symptoms, and conditions are present 4 weeks or more after the initial phase of infection; may be multisystemic; and may present with a relapsing–remitting pattern and progression or worsening over time, with the possibility of severe and life-threatening events even months or years after infection.
 National Institute for Health and Care Excellence	Ongoing symptomatic COVID-19: Signs and symptoms of COVID-19 from 4 weeks up to 12 weeks. Post-COVID-19 syndrome: Signs and symptoms that develop during or after an infection consistent with COVID-19 continue for more than 12 weeks and are not explained by an alternative diagnosis. It usually presents with clusters of symptoms, often overlapping, which can fluctuate and change over time and can affect any system in the body.

Figure 2.6: Definitions of long-COVID (created in Biorender.com; Definitions adapted from National Academies of Sciences, 2024).

This consensus report provided the many manifestations of long-COVID (single or multiple symptoms such as shortness of breath persistent fatigue, difficulty concentrating, light-

headedness etc.) and diagnosable conditions (single or multiple, such as postural orthostatic tachycardia syndrome (POTS), dysautonomia, hypoxemia, migraine, arrhythmias etc.), and made mention that the presence of pre-existing health conditions can exacerbate or present as new conditions. Although this consensus report provides the groundwork for long-COVID definitions, the committee noted that it is still a working definition, and that reconsideration of the definition will be made as new evidence regarding the condition emerges (National Academies of Sciences et al., 2024).

2.3 Respiratory System and Long-COVID

The impact of COVID-19 on the pulmonary system in hospitalized patients remains the most studied area of the impact of COVID-19 in the literature compared to other systems, largely because of the disease primarily affects the respiratory system. It often begins with symptoms similar to the common cold or flu and can progress to pneumonia, which is a typical lung disease (Longobardi et al., 2022).

The primary function of the respiratory system is gas exchange between the atmosphere and the blood to ensure blood gas homeostasis (i.e., arterial oxygen and carbon dioxide) (Lucangelo et al., 2008). For healthy individuals, breathing often seems like a simple, instinctive action that requires little thought. However, despite its apparent simplicity, breathing is both voluntary and programmed and requires complex and coordinated efforts from the respiratory muscles and the brain's control centers to facilitate gas exchange between inspired air and the circulatory system, that is, the removal of carbon dioxide (CO₂) from the body and the supply of oxygen (O₂) to the blood (Lucangelo et al., 2008).

The lung is a highly vascularized structure and contains approximately 600 million alveoli to facilitate efficient gas exchange. This alveolar tissue receives the largest blood supply, and its

diffusion distance remains relatively constant, even throughout levels of physical activity. The alveoli also contain a surfactant that is secreted to reduce surface tension, prevent alveolar collapse at end-expiration, modulate immune response, and interact with and prevent the spreading of pathogens (Han & Mallampalli, 2015; Lucangelo et al., 2008).

Ventilation in the alveoli – the flow of air into and out of the alveoli – and perfusion – the amount of blood flow to the capillaries – play important roles in oxygen delivery and utilization (Cloutier, 2019). Each alveolus has an individual level of ventilation and perfusion, referred to as the ventilation-perfusion ratio (V/Q ratio). This ratio depends on factors such as an alveolar capillary blood volume, body position (e.g. lying down versus standing), and gravity, in that, in an upright position, ventilation per unit of blood volume is lower in the upper lung regions and higher in the lower regions (Cloutier, 2019).

The SARS-CoV-2 virus is most commonly found in the bronchoalveolar lavage fluid, sputum, and saliva of infected individuals, and it primarily spreads through saliva droplets when individuals engage in activities that generate aerosols, such as talking, sneezing, exercising, among others (Liu et al., 2020; Parasher, 2021; Zhang et al., 2020). The sinonasal cavity serves as a major site of SARS-CoV-2 infection and viral shedding due to high expression levels of ACE-2 receptors, which serve as a host receptor for the virus. ACE-2 expression is also highest in type II alveolar cells, which leads to alveolar lung disease, acute respiratory distress syndrome (ARDS), and diffusion alveolar damage as dead cells and protein of damaged lung tissues collect around the alveoli and disrupts gas exchange (Davis et al., 2023; Diab et al., 2021; Osuchowski et al., 2021).

Dyspnea, also known as shortness of breath, is one of the most reported symptoms in individuals with long-COVID (Bliddal et al., 2021). The majority of severely affected COVID-

19 patients develop acute respiratory distress syndrome (ARDS), a severe type of acute lung injury marked by hypoxemia and the presence of ground glass nodules in X-ray and MRI images (Batah & Fabro, 2021). This syndrome impacts lung diffusion capacity by causing damage to the alveolar-capillary barrier, leading to increased vascular permeability, and reducing lung compliance and aerating tissue size, compromising gas exchange and leading to hypoxemia (Batah & Fabro, 2021). Beyond the acute phase of the disease, diffusion capacity limitation has been observed as a common complication in the cardiopulmonary system of COVID-19 survivors, persisting four months after the onset of the disease (Huang et al., 2020; Mo et al., 2020). The proposed mechanism for these and other persistent dysfunctions and symptoms related to long-COVID is a hyperinflammatory response known as the “cytokine storm” (Jammoul et al., 2023; Montazersaheb et al., 2022).

A cytokine storm is an intense inflammatory immune response where the body rapidly releases a large number of cytokines, such as interleukin (IL)-6, IL-17, and tumor necrosis factor (TNF), into the bloodstream (Montazersaheb et al., 2022). While cytokines are essential for normal immune function, an excessive release can be harmful, causing symptoms like high fever, inflammation (redness and swelling), severe fatigue, and nausea. In severe cases, a cytokine storm can be life-threatening and may lead to multiple organ failure (Montazersaheb et al., 2022). Figure 2.7 displays the mechanism leading to a cytokine storm.

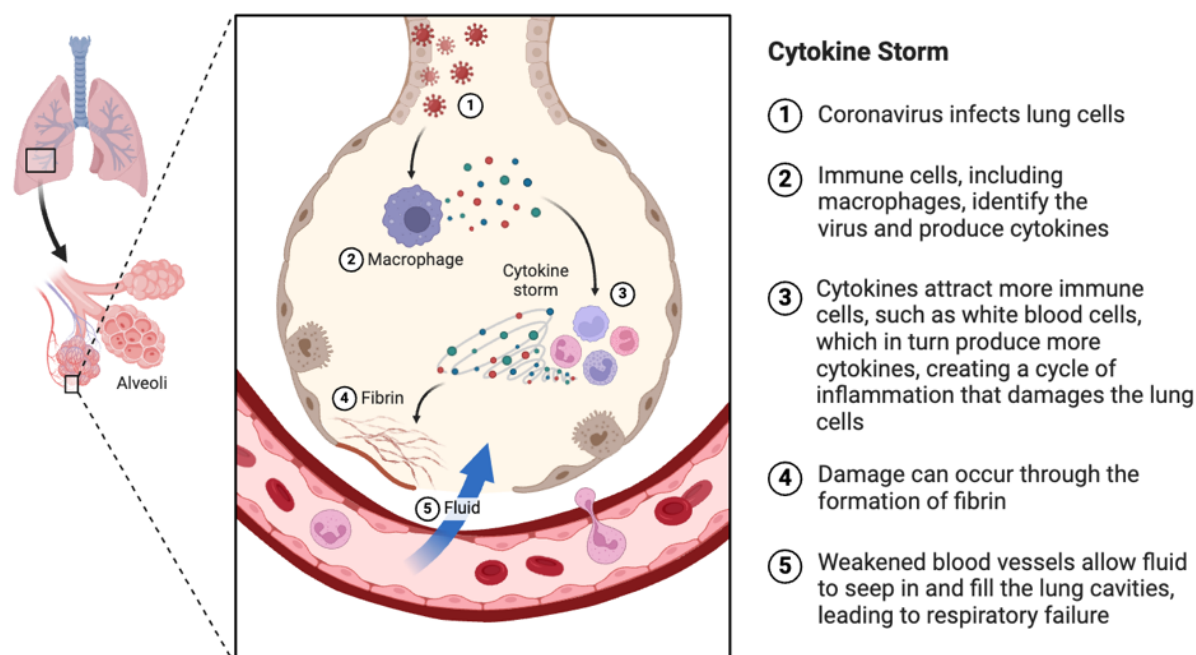


Figure 2.7: Mechanism leading to a cytokine storm (adapted from Biorender.com).

Lung dysfunction following SARS-CoV-2 infection is often persistent, particularly in critically ill patients (Batah & Fabro, 2021; Montazersaheb et al., 2022). Studies show that upon hospital discharge, a significant proportion of critically ill COVID-19 patients have persistent lung dysfunction. Studies reported that diffusion capacity, diminished gas-blood exchange function in these patients, and restrictive ventilatory defects were common abnormalities in discharged COVID-19 survivors, particularly those with severe cases (Li et al., 2021; Mo et al., 2020). Van Den Borst et al. (2020) further supported these findings, revealing that 42% of discharged patients ($n = 124$) had reduced lung diffusion capacity, and 91% had residual pulmonary parenchymal abnormalities (Van Den Borst et al., 2021). A study also reported that after testing using pulmonary function and respiratory muscle strength testing measurements, 55% of these patients ($n = 22$) had impaired lung function in the form of showing a restrictive pattern and an altered diffusing lung capacity, three months after ICU discharge, with the

severity of radiologic lung involvement at admission being a key factor (Truffaut et al., 2021).

These studies collectively suggest that ongoing lung dysfunction is a common issue in critically ill COVID-19 patients at hospital discharge. Figure 2.8 shows a comparison of healthy and affected alveoli by SARS-CoV-2.

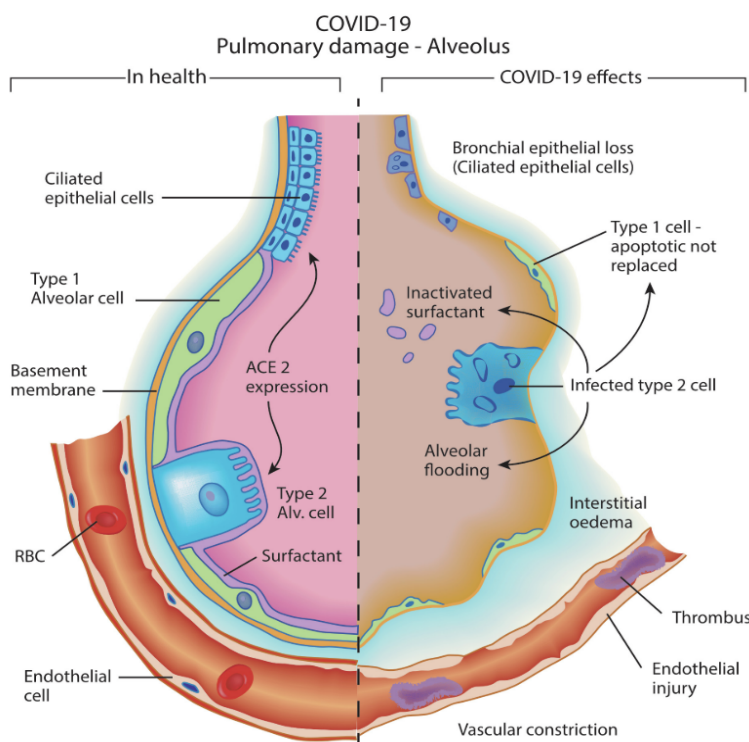


Figure 2.8: Comparison of a healthy alveolus and an alveolus affected by SARS-CoV-2 (adapted from Gan et al., 2020).

Abnormalities in lung imaging and pulmonary function tests generally align with the severity of the initial SARS-CoV-2 infection, and impairment in diffusion capacity is a consistent abnormality (Huang et al., 2020; Mo et al., 2020). Objectively abnormal lung function tests tend to persist six months to one year after hospitalization, and longitudinal imaging at eight months post-discharge shows abnormalities in one-third of cases, and at one year, 25% exhibit ground glass opacities and subpleural lesions (Huang et al., 2020). While most hospitalized

patients' pulmonary function tests return to normal at one year, up to one-third still experience exertional dyspnea (Huang et al., 2020). However, limited research is available regarding understanding the mechanisms of organ dysfunction in hospitalized COVID-19 patients and the long-term effects on the lungs of nonhospitalized COVID-19 patients (Parhizgar et al., 2023). However, persistent symptoms like breathlessness in both hospitalized and non-hospitalized individuals might be influenced by factors extending beyond the pulmonary system.

2.4 Cardio-autonomic Systems: Autonomic Influences on the Cardiovascular System

The cardiovascular system consists of the heart, vessels, and blood, and is essential for maintaining homeostasis and supporting cellular function. The three main functions of this system include: i) transporting materials (nutrients, oxygen, water etc.) entering the body to utilization sites, ii) transporting materials between cells within the body (antibodies, hormones, stored nutrients etc.) and iii) transporting materials from cells to sites of elimination (e.g., waste, carbon dioxide, heat) (Pappano & Wier, 2019).

The autonomic nervous system (ANS) is a branch of the nervous system which plays a crucial role in maintaining blood pressure (Pappano & Wier, 2019). The ANS is divided into two major subsections, the parasympathetic and sympathetic nervous systems, which are critical in mediating the cardiovascular, thermoregulatory, and metabolic changes necessary for internal homeostasis during energy expenditure (Fisher et al., 2015; Pappano & Wier, 2019). These physiologic adjustments mediated by the ANS are also required to achieve maximal aerobic capacity during exercise performance (Fisher et al., 2015; Pappano & Wier, 2019). Figure 2.9 represents the divisions of the nervous system.

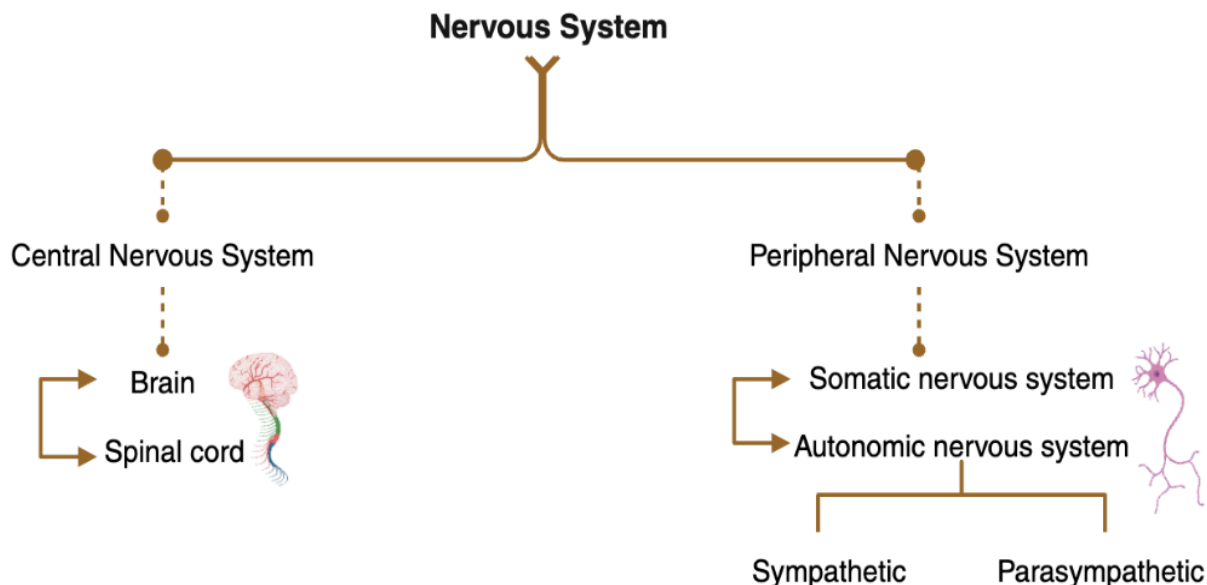


Figure 2.9: Divisions of the nervous system (created in Biorender.com).

The regulation of cardiac functions is influenced by both neural and humoral factors. The neural regulation is mediated by the autonomic nervous system, which consists of the parasympathetic and sympathetic branches. The cardiovascular system is influenced by the nervous system to several varying degrees, including an influence on the frequency of heartbeats (heart rate) and the force of contractility (Pappano & Wier, 2019)

Heart rate variability (HRV) is an indirect measure of autonomic nervous system activity in the cardiac muscle (Koenig & Thayer, 2016; Shaffer et al., 2014). In a healthy individual, there is a dynamic balance between sympathetic and parasympathetic influences that governs overall body function. The sinoatrial (SA) node, the heart's dominant pacemaker, spontaneously depolarizes and generates action potentials that propagate electrical activity throughout the atria and ventricles. This activity can be recorded using an electrocardiogram (ECG) device (Pappano & Wier, 2019).

Both the sympathetic and parasympathetic branches of the autonomic nervous system are active simultaneously, influencing heart activity. The degree of their influence is modulated by the cardiovascular control center in the medulla of the brainstem, which integrates sensory information from proprioceptors, chemoreceptors, and baroreceptors, as well as input from the cerebral cortex and limbic system (Shaffer et al., 2014). At any moment, the heart rate reflects the combined effects of parasympathetic nerves, which dominate during rest, and sympathetic nerves, which become more active during stress or fight-or-flight responses (Shaffer et al., 2014)..

Humoral factors also contribute to the regulation of cardiac functions, such as cardiac output (Ghosh & Pandit, 2019). The influence of epinephrine and norepinephrine from the adrenal medulla serves as an extrinsic control of cardiac output, modulating sympathetic and parasympathetic nerve activity through influence on the veins (to increase venous return via vasoconstriction), the ventricles (to increase contractility) and on the SA node (to increase heart rate) (Ghosh & Pandit, 2019). Figure 2.10 summarizes the humoral control of cardiac output.

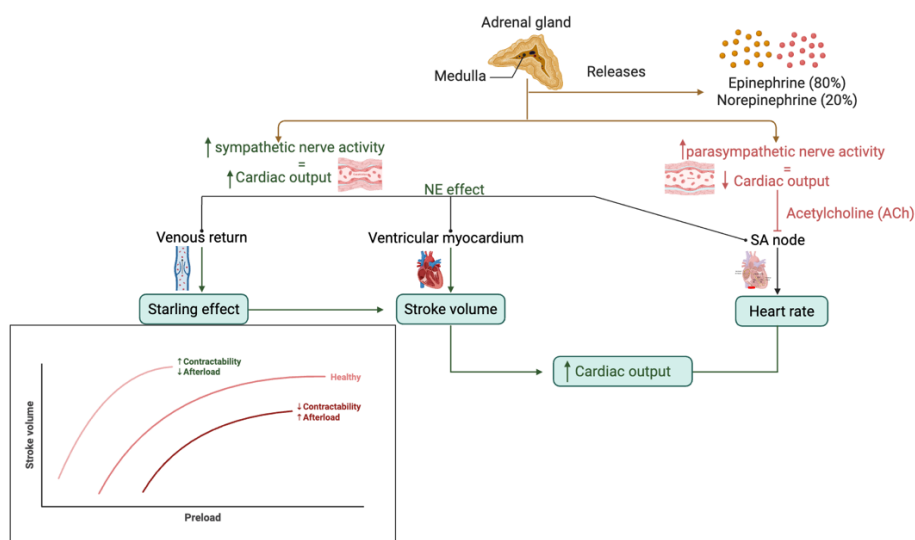


Figure 2.10: Humoral control of cardiac output (created in Biorender.com).

Although long-COVID is more commonly associated with and talked about in the sphere of the respiratory system due to the nature of the virus, emerging research indicates that the SARS-CoV-2 virus also leads to dysfunctions in the cardiovascular system (Brown et al., 2022; Parhizgar et al., 2023). The severity of the disease is linked to the presence of ACE-2 receptor on cardiac and vascular endothelial cells, which implicates the elevation of markers indicating endothelial activation and circulating endothelial cells (Davis et al., 2023; Osuchowski et al., 2021). The elevated markers subsequently have the potential to initiate a "cytokine storm" and are associated with elevated oxidative stress, which negatively affects the cardiovascular system (Davis et al., 2023; Osuchowski et al., 2021).

Additionally, other studies have reported myocarditis and myocardial inflammation in individuals with long-COVID, despite no previous history of myocarditis or any other heart disease (Brown et al., 2022; Parhizgar et al., 2023). Furthermore, irrespective of whether COVID-19 patients were hospitalized or not, studies show continued abnormal cardiac MRI results and evidence of diastolic dysfunctions (Linschoten et al., 2020; Xie et al., 2022). Myocarditis persisted 6 months after discharge in hospitalized patients, with more noticeable tissue damage in those who experienced a more severe acute phase (Xie et al., 2022). Huang et al.'s (2020) study identifying cardiac involvement in patients recovered from COVID-19 shows abnormalities and dysfunctions in the heart's right ventricle, implicating cardiac functions (e.g., ventricular tachycardia). However, it is crucial to note that the variations in the prevalence of cardiac involvement across studies could be caused by variations in participant selection (e.g., recruiting from a cardiac rehabilitation clinic, recruiting participants with persistent cardiac symptoms vs. those with persistent respiratory symptoms, and criterion researchers use for

categorizing patients as long-COVID patients (using the WHO definition vs. NIH definition)) (National Academies of Sciences et al., 2024).

2.5 Orthostatic Stress Testing

The assessment of how the body's physiological systems respond to changes in posture, particularly during transitions from supine to sitting or standing, as well as the diagnosis of orthostatic intolerance, is often conducted using orthostatic stress tests (McJunkin et al., 2015; Monaghan et al., 2022; O'Connell et al., 2015; Ricci et al., 2015). These tests are instrumental in clinical and research settings and are commonly used to identify dysfunctions and understand physiological adaptations in conditions such as orthostatic hypotension and postural tachycardia syndrome (Hoyt et al., 2021; Ricci et al., 2015; Romero-Ortuno et al., 2011).

Although a variety of tests exist to assess autonomic and cardiovascular dysfunction related to orthostatic intolerance, such as the National Aeronautics and Space Administration (NASA) lean test (NLT), Deep breath testing, and Valsalva maneuver, the most commonly used protocols are the active stand test and the head-up tilt-table test (Lee et al., 2020; Low et al., 2013; Plash et al., 2013). Given their ability to detect abnormalities in autonomic and cardiovascular regulation during postural changes, these tests have become particularly important in assessing long-COVID, as symptoms of dysautonomia, orthostatic intolerance, and postural tachycardia syndrome (POTS) are increasingly reported (Blitshteyn & Whitelaw, 2021; Fedorowski et al., 2023; Hira et al., 2025).

The Physiology of Postural Changes

For humans, everyday activities often present an orthostatic stress, as changes in posture cause a shift of blood away from the chest region. For example, individuals perform ~50 – 60 sit-to-stand transitions each day (Bohannon, 2015; Dall & Kerr, 2010). This gravitational shift,

depending on the type of orthostatic stress (e.g. active standing, head-up tilt, etc.) results in approximately 300-500 mL of blood pooling in the venous capacitance systems below the diaphragm (Freeman et al., 2011; Ricci et al., 2015). In healthy individuals, the decrease in central blood volume (i.e., blood available to the cardiac ventricles) occurs within the first 10 seconds and activates compensatory mechanisms that are crucial for regulating blood pressure and preventing syncope (Smit et al., 1999; Wieling & Groothuis, 2012).

Upon standing, gravity causes an immediate pooling of blood in the venous capacitance vessels of the lower body. This retribution leads to a rapid decrease in venous return, arterial blood pressure, stroke volume, and subsequently cardiac output through the Frank-Starling mechanism (Smith et al., 1999). In healthy individuals, these rapid changes are detected by arterial baroreceptors located in the aortic arch and carotid sinuses, as well as low-pressure volume cardiopulmonary receptors located within the atria, ventricles, and pulmonary vasculature (Smith et al., 1999; Wieling & Groothuis, 2012). These receptors maintain blood pressure through circulatory and renal regulation (Armstrong et al., 2023; Sata et al., 2018). The reduced stretch of the artery wall sensed by these receptors leads to a decrease in afferent firing, prompting an increase in sympathetic outflow and a withdrawal of parasympathetic (vagal) tone through the brainstem's vasomotor centers within seconds of standing (Armstrong et al., 2023). The resulting adjustments trigger initial short-term compensatory mechanisms to counteract the drop in blood pressure, resulting in an increase in heart rate, myocardial contractility, and systemic vascular resistance (baroreceptor mediated vasoconstriction) restoring blood pressure within 15-30 seconds after standing (Armstrong et al., 2023; Wieling et al., 2022; Wieling & Groothuis, 2012). Additionally, the cardiopulmonary receptor stimulates sympathetic outflow to the sinus node, contributing to the tachycardic response.

However, if an orthostatic stress is prolonged, an increase in heart rate (frequency of heartbeat) and contractility (force of contraction) alone is insufficient to counteract the drop in blood pressure. Therefore, the renin-angiotensin-aldosterone system (RAAS) is gradually activated over time. The cardiopulmonary receptors contribute to this response by sensing the reduced blood volume and triggering hormonal pathways that increase renal sodium and water reabsorption (Armstrong et al., 2023; Sata et al., 2018). Concurrently, increased sympathetic outflow stimulates the vasoconstrictor centers in the medulla, initiating a cascade that increases angiotensin II production (Sata et al., 2018). This mediates vasoconstriction and maintains longer-term blood pressure regulation by increasing blood volume and maintaining mean arterial pressure (Armstrong et al., 2023; Wieling et al., 2022; Wieling & Groothuis, 2012a).

In conjunction with the RAAS, skeletal muscle contraction, facilitated by the skeletal muscle pump, helps regulate blood pressure through postural sway. This mechanism mitigates the effects of orthostatic intolerance by helping improve venous return (Wieling et al., 2022; Wieling & Groothuis, 2012). Figure 2.11 illustrates a schematic representation of the mechanisms involved in postural transitions.

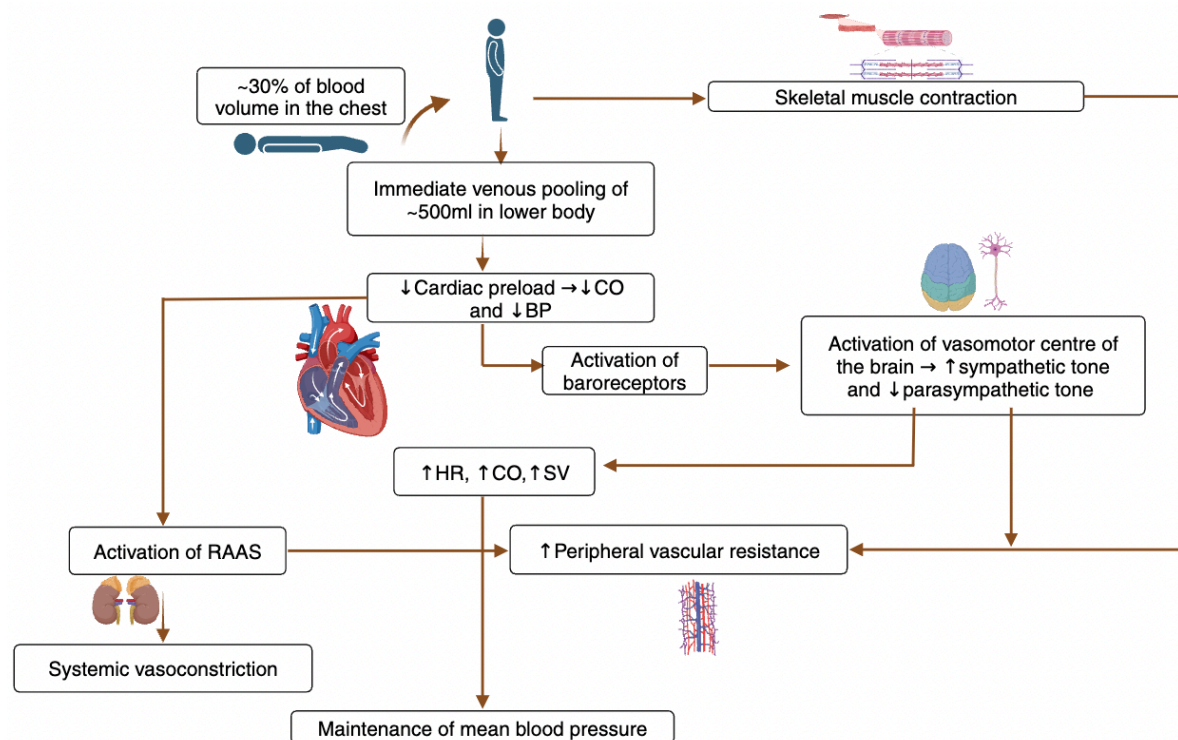


Figure 2.11: Mechanism behind postural change (created in Biorender.com).

2.6 Cardio-autonomic System and Long-COVID: Dysautonomia, Orthostatic Intolerance, and Postural Orthostatic Tachycardia Syndrome

Dysautonomia refers to dysfunction in the autonomic nervous system (ANS) and includes disorders like postural orthostatic tachycardia syndrome (POTS), neurocardiogenic syncope, and orthostatic intolerance (OI) (Fedorowski et al., 2023; Novak et al., 2022). Orthostatic intolerance, a common symptom of dysautonomia, is characterized by a reduced ability to sustain an upright posture (Ricci et al., 2015). This condition poses a physiological challenge as it requires a coordinated neuro-cardiovascular response to maintain adequate blood pressure, heart rate, and organ perfusion (O'Connell et al., 2015; Ricci et al., 2015).

A specific type of orthostatic intolerance, orthostatic hypotension (OH), occurs when the cardiovascular system fails to compensate for reduced venous return upon standing (Ricci et al.,

2015). Although this condition is often associated with frailty in older adults, recent research shows that people experiencing long-COVID also experience this condition, leading to symptoms like dizziness, lightheadedness, blurry vision, increasing the risk of syncope and falls, and consequently, the risk of fall injuries such as, fractures, head injuries, among others (Blitshteyn & Whitelaw, 2021; Ormiston et al., 2022; Vernon et al., 2022).

The most recent consensus statement in 2011 defined orthostatic hypotension as a decrease of at least 20 mmHg in systolic blood pressure (SBP) or at least 10 mmHg in diastolic blood pressure (DBP) within 3 minutes of assuming an upright position (Freeman et al., 2011). Due to the influence of gravity on vascular systems, orthostatic hypotension is a natural response during postural changes, but in healthy individuals, orthostatic stabilization is typically reached within 1 minute (Ricci et al., 2015). However, in individuals with comorbidities such as dysautonomia, as in the case of long-COVID, three types of orthostatic hypotension may occur based on temporal changes in blood pressure. These types are presented in table 2.1 and illustrated in Figure 2.12.

Table 2.1. Classification of Orthostatic Hypotension (Freeman et al., 2011; Moya et al., 2009)

Type	Definition
Initial OH	Rapid BP decrease ($> 40\text{mmHg}$ SBP and/or $> 20\text{mmHg}$ DBP) within 0-30s of standing.
Classical OH	Decrease of at least 20 mmHg SBP and/or 10 mmHg DBP within 30-180 s of active standing or head-up tilt to at least 60° .
Delayed OH	Gradual progressive drop in blood pressure of at least 20 mmHg SBP and/or 10 mmHg DBP or at least 15 mmHg in individuals with hypertension between 3 and 45 minutes.

OH: orthostatic hypotension; BP: blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; HUT: head-up tilt.

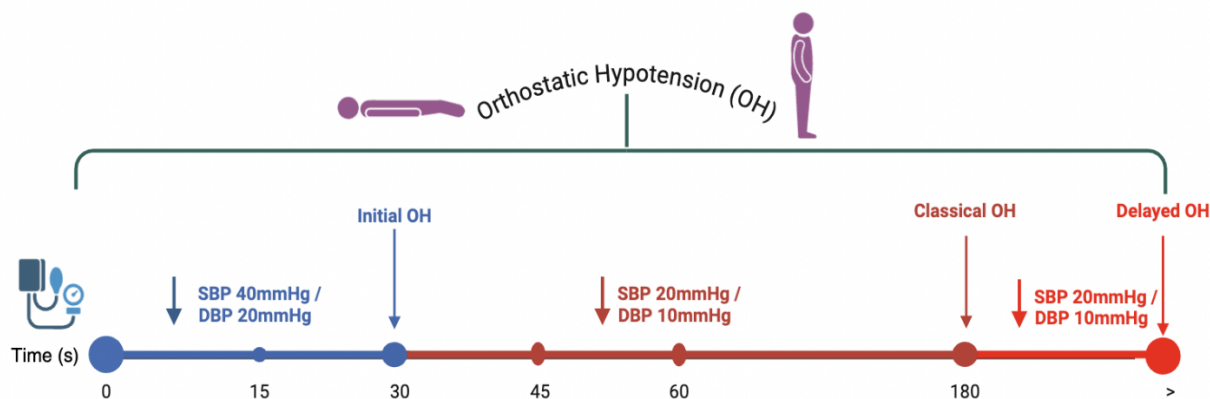


Figure 2.12: Orthostatic hypotension (created in Biorender.com).

Postural Orthostatic Tachycardia Syndrome (POTS), a form of dysautonomia, has been reported in 9% to 61% of individuals with long-COVID (Blitshteyn & Whitelaw, 2021; Campen & Visser, 2022; Ormiston et al., 2022), is characterized by a heart rate increase of 30 beats per minute (bpm) or more within 10 minutes of assuming an upright position, and this occurs without orthostatic hypotension (Freeman et al., 2011). While its underlying causes are not thoroughly understood, the commonly proposed mechanism for POTS in people experiencing long-COVID is associated with symptoms of lightheadedness and dizziness, which are attributed to a reduced orthostatic cerebral blood flow (cerebral hypoperfusion) (Bliddal et al., 2021; Blitshteyn & Whitelaw, 2021; Ormiston et al., 2022). However, inconsistencies in results arise in existing studies examining POTS (Blitshteyn & Whitelaw, 2021; Monaghan et al., 2022).

Monaghan et al.'s (2022) study on orthostatic intolerance (OI) among older adults with long-COVID showed that only 13% (7 participants) of the 53 OI group exhibited a heart rate increase of >30 bpm upon standing. Intriguingly, among this subgroup, only 2% (1 participant) reported awareness of the heightened heart rate and accompanying sensations of light-

headedness despite all seven individuals exhibiting observable physiological changes in heart rate. In contrast, Blitshteyn et al.'s (2021) case study on 20 individuals with long-COVID reported a substantially higher prevalence of POTS. In their cohort, 75% of participants ($n = 15$) exhibited POTS during orthostatic intolerance stress tests.

The pathophysiology of autonomic dysfunction in individuals with long-COVID also remains highly speculative as dysautonomia originally can arise from impairment in various areas (viral invasion of autonomic centers or hypothalamic impairment), which could either be neuronal or hematogenous (Davis et al., 2023; Serviente et al., 2022). However, the exaggerated inflammatory response (often referred to as a cytokine storm) triggered by SARS-CoV-2 infection is often cited as a contributing mechanism underlying autonomic dysfunction in individuals with long-COVID (Acosta-Ampudia et al., 2022; Chen et al., 2024; Rojas et al., 2022). This interplay between inflammation and the sympathetic nervous system (SNS) overactivation creates a chronic inflammation feedback loop, whereby the exaggerated inflammatory response triggers SNS activation, which then triggers a pro-inflammatory response, leading to an increased risk for cardiovascular diseases and subsequently affecting other systems (Acosta-Ampudia et al., 2022).

In relation to this interplay, some studies have shown that individuals with long-COVID tend to show increased sympathetic activity at rest compared to individuals without long-COVID (Shah et al., 2022; Stute et al., 2021), although some others have reported parasympathetic hyperactivity as measured by heart rate variability (Ferreira et al., 2024; Marques et al., 2023; Zacher et al., 2023). However, it is agreed upon that COVID-19 affects the sympathovagal balance of the autonomic nervous system, and the variability that exists in the literature might be attributed to factors such as the timing of post-infection autonomic control assessments, age of

study participants, disease severity, and general health of individuals prior to SARS-CoV-2 infection (Ferreira et al., 2024; Osuchowski et al., 2021). Figure 2.13 shows the relationship between autonomic nervous system and heart rate variability (adapted from (Marques et al., 2023)).

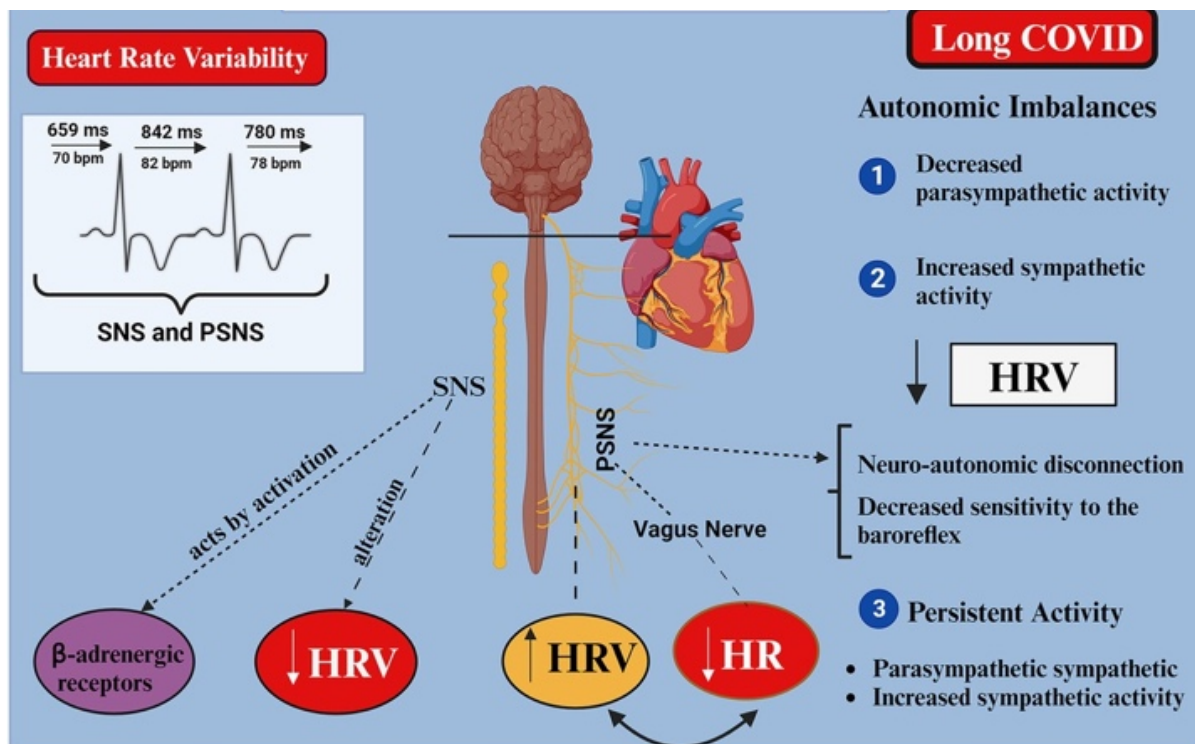


Figure 2.13: Autonomic nervous system and heart rate variability (adapted from (Marques et al., 2023)).

This discrepancy in study characteristics and conflicting findings emphasize the complexity of cardiovascular dysautonomia manifestations in long-COVID, highlighting the need for further research to clarify the underlying mechanisms within this population.

2.7 Gaps in Literature, Emerging Areas, and Problem Statement

The multi-system impact of COVID-19, particularly in relation to long-COVID, emphasizes the debilitating nature of this condition. Despite growing recognition of long-COVID

as a condition affecting multiple physiological systems, the cardio-autonomic abnormalities underlying many of its key reported symptoms remain poorly understood. Although research on long-COVID is still relatively in its early stages, it is important to acknowledge the heterogeneity in existing evidence and recognize the limitations of current studies, where both measured and unmeasured factors may confound result interpretation.

While existing studies have established that a subset of individuals with long-COVID experience symptoms consistent with dysautonomia, including elevated rates of conditions such as POTS and OH (Basaca et al., 2025; DePace & Colombo, 2022; Vernon et al., 2022), much of the current research still relies on manual measurements or subjective symptom reporting, and studies using continuous, dynamic physiological data remain limited. Additionally, although biomarkers and immunological phenotypes have been explored, there is a notable lack of research assessing early compensatory cardiovascular responses (e.g., within the first 30-60 seconds of standing). Most studies to date have also focused on hospitalized or severely affected long-COVID populations, leaving a gap in understanding how cardio-autonomic dysfunction presents in community-dwelling or less severely affected individuals, who represent a larger proportion of those with long-COVID (Davis et al., 2023; Saheb Sharif-Askari et al., 2024). Emerging research points toward the value of short, non-invasive autonomic screening protocols for identification of cardiovascular dysfunction in the long-COVID population, and active stand tests present a feasible method for use in outpatient and research settings, as they also closely mimic daily postural transitions.

Therefore, the systematic review of this thesis (in chapter 4) aims to synthesize existing literature on the use of an active standing test to assess physiological responses, while the cross-sectional study (in chapter 5) seeks to address key methodological and physiological gaps using a

continuous, beat-by-beat active standing protocol to explore short-term cardiovascular compensatory responses in individuals with long-COVID. By assessing cardiovascular dynamics across early and late postural transition phases, the aim is to contribute to a more integrated understanding of cardiovascular responses in this population.

CHAPTER 3: GENERAL METHODOLOGY

The following sections present the general methodology of the systematic review and the observational cross-sectional study. Detailed methodology would be included in Chapters 4 and 5, respectively.

3.1 Systematic review

The systematic review aimed to assess the current state of evidence on using active standing orthostatic stress test to assess cardiovascular, autonomic, and respiratory responses in individuals with long-COVID, and the methodological limitations or gaps in this emerging body of literature. The systematic review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 statement (Page et al., 2021), and it was registered in the PROSPERO international database (CRD-42024615872). The methodology included the study design, eligibility inclusion and exclusion criteria, information sources and search strategy, study selection, data extraction, quality and risk of bias assessment, data synthesis and analysis. To minimize repetition, specific details are provided in Chapter 4.

3.2 Observational cross-sectional study

The observational cross-sectional study aimed to report the frequency of occurrence of OH and OI symptoms in long-COVID (LC) participants, characterize and compare the short-term cardiovascular compensatory responses to an active standing orthostatic stress test between individuals experiencing LC and non-long-COVID with OH (NLC_{OH}) and non-long-COVID with normal blood pressure response (NLC_{NBPR}) individuals. The study was performed as part of a larger research project examining the cardiovascular, autonomic, respiratory and brain oxygenation responses to active standing orthostatic stress tests in individuals with long-COVID. The study was reported following the Strengthening the Observational Studies in Epidemiology

(STROBE) statement guidelines (Von Elm et al., 2007). This study took place in the Cardiorespiratory and Physiology of Exercise Research Laboratory in the Applied Research Centre at the University of Manitoba, Winnipeg, Manitoba, Canada, and was approved by the Research Ethics Board 1 (REB-1), University of Manitoba Fort Gary Campus (HE2024-0364). To minimize repetition, the comprehensive details needed are presented here, with a concise summary included in the manuscript in chapter 5.

3.2.1 Initial Assessment Protocol

Participants arrived at the laboratory in a fasted state (at least 2 hours before). Upon arrival, participants were seated for approximately 15 minutes to allow physiological stabilization. During this period, detailed verbal information about the testing procedure was provided. Participants were then asked to review and sign an informed consent form and were encouraged to ask questions at any point before data collection started. Participants then completed the short-form Depaul Symptom Questionnaire (DSQ-SF), rating the severity and frequency of symptoms over the past six-month period. The purpose of the questionnaire was to assess symptomology related to myalgia encephalomyelitis/chronic fatigue syndrome (ME/CFS), a condition that shares clinical similarities with long-COVID, particularly in relation to post-exertional malaise (PEM), fatigue, cognitive dysfunction, among others (Buntić et al., 2024; Komaroff & Lipkin, 2023; Wold et al., 2024).

Following the rest period, baseline physiological measurements were obtained, including BP, HR, and oxygen saturation (SPO₂). These pre-test measures ensured that participants met safety eligibility criteria based on the Canadian Society for Exercise Physiology (CSEP) guidelines, which include a BP reading of $\leq 160/90$ mmHg, $HR \leq 100$ bpm¹ and $SPO_2 \geq 92\%$ (Canadian Society for Exercise Physiology, 2017). During this measurement period, participants

were instructed to sit upright with both feet flat on the floor and their right arm resting on the table, palm facing upward (Canadian Society for Exercise Physiology, 2017). BP and HR were assessed using a digital reusable cuff (Welch Allyn Flexiport™, Welch Allyn Inc., New York, USA), while oxygen saturation was measured using a finger pulse oximeter (Ohmeda Biox 2700, Biox Technology Inc., Boulder, Colorado). If a participant's values exceeded any of the cut-off thresholds, measurements were repeated after an additional 5 minutes of seated rest. Participants who continued to exceed the safety thresholds were ineligible to participate in the study. They were advised to consult their family physician and were provided with the CSEP's Physician Physical Activity Readiness Clearance form (Canadian Society for Exercise Physiology, 2017). One recruited participant was deemed ineligible to participate due to BP readings above cut-off values, and due protocol was followed.

Demographic questions, including questions of sex assigned at birth, date of birth, age, and relating to SARS-CoV-2 infection (e.g., most recent date of infection, severity of infection, hospitalization status) were collected to characterize the sample. Anthropometric parameters were also measured, including height using a Seca 213 portable stadiometer (Seca Deutschland, Hamburg, Germany), body mass using an InBody scale (InBody Canada Co. Ltd., Ontario, Canada), and waist and hip circumference using a tape measure. Whole-body segmental bio-electrical impedance was also assessed with the InBody-270 body composition analyzer (InBody Canada Co. Ltd., Ontario, Canada), which measures an individual's total body water, dry lean mass, skeletal muscle mass, body fat mass, body mass index, lean muscle mass, and percentage of body fat. Medication use was also collected, although our participants were asked to abstain from medications 24 hours prior to their test date if applicable.

3.2.2 Study Protocol

All participants underwent two active postural transition tests: (a) Sit-to-Stand (SS) and (b) Lie-to-Stand (LS), which represent a low and high orthostatic stress challenge, respectively. Prior to data collection, participants were given a brief familiarization session, particularly for the lie-to-stand transition, to demonstrate the assistance provided by two research assistants during the transition period. This assistance was provided to minimize the use of upper body muscles, which could interfere with the electrocardiogram (ECG) signal quality placed on participants' chests. This was also done to prevent participants from using their hands to push into a seated position, as this could potentially disrupt readings by the finger photoplethysmograph cuff. To mitigate anticipatory physiological responses, research assistants remained nearby throughout the duration of the testing and only approached the participant approximately 5 seconds before the transition. Participants were also instructed to stand still during up to 7 minutes, minimizing any sways or movements (Finucane et al., 2019). After the test, participants were asked if they experienced any symptoms relating to orthostatic intolerance (e.g. light-headedness, dizziness) upon standing.

The postural transitions were conducted as follows:

- Sit-to-stand (SS): Participants sat upright in a chair for 5 minutes before standing for up to 7 minutes (Figure 3.1A).
- Lie-to-stand (LS): Participants lay supine for 10 minutes and then transition into standing for up to 7 minutes (Figure 3.1B).

The order of the protocols was randomized and counterbalanced to control for potential order and carryover effects. A 10-minute seated break period was also provided in between conditions.

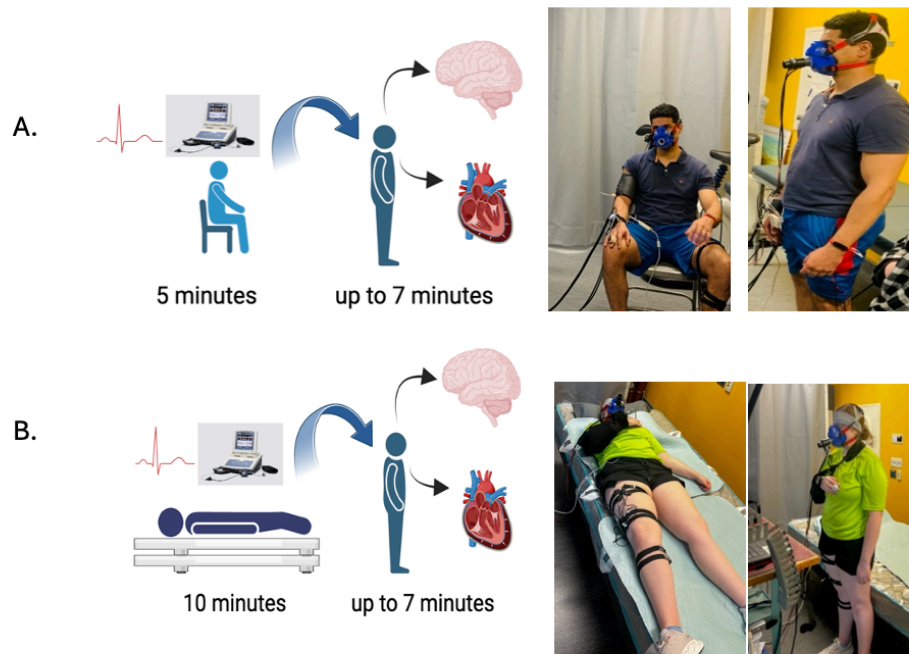


Figure 3.1: Active standing protocols. A. Sit-to-Stand (SS) B. Lie-to-stand (LS)

3.2.3 Data Collection

Cardiovascular variables of interest – systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), systematic vascular resistance (SVR), heart rate (HR), cardiac output (CO) and stroke volume (SV) – were continuously monitored using the Finometer (Finapres Medical System, Colorado Springs, CO, USA) integrated into LabChart 8 software (LabChart 8, ADInstruments, Colorado Springs, CO, USA). The non-invasive blood pressure (NIBP) Finometer uses finger cuff photoplethysmography to detect arterial pressure signals by emitting light into the tissue and analyzing reflected light to estimate beat-by-beat changes in blood volume (Imholz et al., 1990; Petersen et al., 1995; Waldron et al., 2018).

Beat-by-beat HR calculations were derived using a 3-lead ECG setup (electrodes placed on the right arm [RA], right leg [RL], and left leg [LL]), recorded through an Octal Bio Amp module (ADInstruments, Finapres Medical System, Colorado Springs, CO, USA). R-waves were

detected in LabChart 8 software, and inter-beat intervals were used to compute HR (in bpm) (LabChart 8, ADInstruments, Colorado Springs, CO, USA). Stroke volume (in mL) was derived from the formula: $SV = (CO \div HR) \times 1000$. MAP was calculated as the average of the arterial pressure waveform over each cardiac cycle using LabChart's cyclic measurement function (LabChart 8, ADInstruments, Colorado Springs, CO, USA). SVR was estimated by dividing MAP by CO ($SVR = MAP \div CO$). Cardiac output was calculated using the model flow algorithm within LabChart (LabChart 8, ADInstruments, Colorado Springs, CO, USA).

Participants were also fitted with a face mask for breath-by-breath respiratory variables monitoring using a metabolic cart (Vynthus™, CPX Metabolic cart, Vyaire Medical Inc., Chicago, USA), and a non-invasive monitoring of cerebral and skeletal muscle (measured at the thigh and calf) total hemoglobin (tHb), oxyhemoglobin (HbO₂), deoxyhemoglobin (HHb), and tissue oxygen saturation (tSO₂) using the Near-Infrared Spectroscopy (NIRS) (OctaMon M and Portalite– Artinis Medical System BV, The Netherlands). However, these variables were collected as part of a larger research project exploring those outcomes on long-COVID and are not reported in Chapter 5.

3.2.4 Data Analysis

Beat-by-beat data of all variables of interest (SBP, DBP, MAP, SVR, HR, CO, SV) was collected and processed using LabChart 8.0 (ADInstruments, LabChart 8, Colorado Springs, CO, USA). The processed data was then saved and transferred to a custom MATLAB application, where the time-alignment marking zero was made to correspond to the start of the transition (MATLAB R2024, MathWorks, Inc., USA).

Preprocessing of cardiovascular signals involved the removal of motion artifacts and noise using a median and low pass filter with a cut-off frequency of 0.05 rad/sample (from scalogram

analysis) was applied, and a 5-second moving average filter was also used to minimize the influence of inter-beats and inter-breath oscillations (van der Velde et al., 2007). Data interpolation was conducted, and data was corrected to address time delays (using a resampling rate of 1Hz). The reference point zero on the x-axis indicated the transition point, with negative values indicating the baseline phase and positive values indicating responses observed after the transition.

Baseline values were determined as an average of the final 120 seconds before standing. Following transition, the lowest values for SBP, DBP, MAP, SVR and the highest values for HR, CO, SV within the first 30 seconds were extracted to capture the initial cardiovascular response. The peak recovery point after the initial drop in each phase was used to define short-term compensatory responses. The phases were specified as follows: phase 1 (0-30 s), phase 2 (30-60 s), phase 3 (60-180 s), and phase 4 (300-420 s). This phased approach is based on typical physiological recovery patterns in healthy adults and established orthostatic hypotension criteria, allowing for the assessment of adaptive responses and differences in cardiovascular regulation (Podoleanu et al., 2009; Romero-Ortuno et al., 2010). Figure 3.2 shows the expected physiological responses during a lie-to-stand orthostatic stress test. Table 3.1 below describes the variables of interest being analyzed.

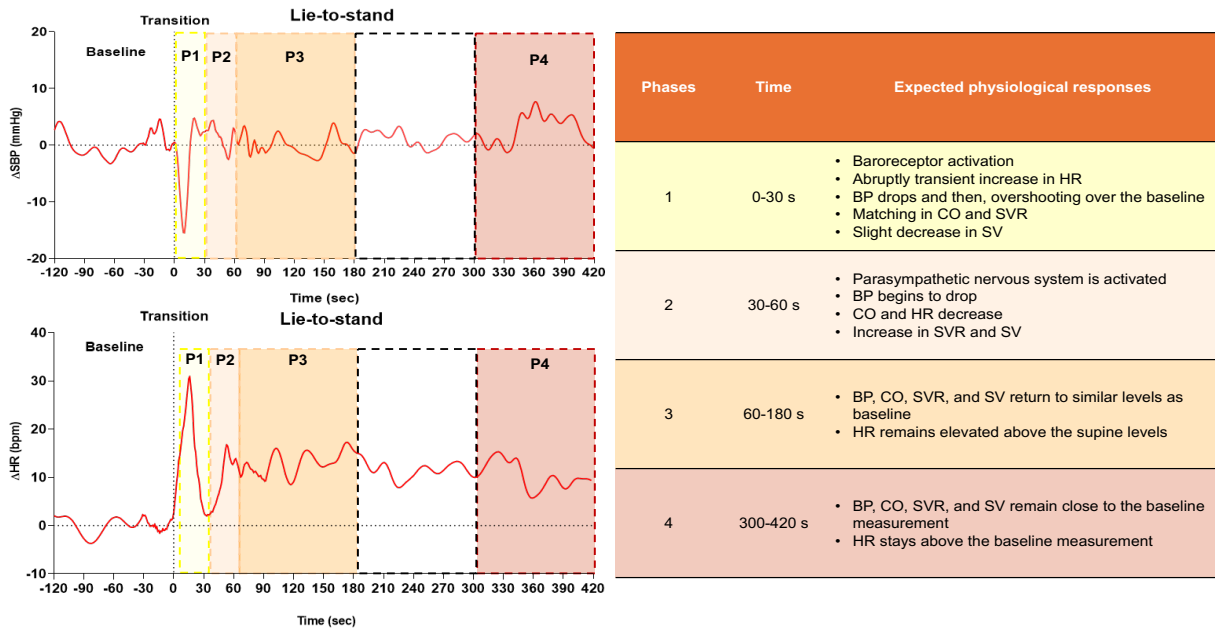


Figure 3.2: Expected physiological responses of short-term SBP (top) and HR (bottom) responses to lie-to-stand divided into 4 phases.

Table 3.1. Cardiovascular measurements across phases in different time intervals.

Variable	Description
Baseline	
SBP and DBP baseline (mmHg)	Average SBP and DBP over 120 seconds before transition
MAP baseline (mmHg)	Average MAP over 120 seconds before transition
SVR baseline (mmHg.min ⁻¹ L ⁻¹)	Average SVR over 120 seconds before transition
HR baseline (bpm)	Average HR over 120 seconds before transition
CO baseline (L.min ⁻¹)	Average CO over 120 seconds before transition
SV baseline (mL)	Average SV over 120 seconds before transition
Amplitude (after transition)	

SBP and DBP amplitude (mmHg)	Difference of baseline SBP and DBP to nadir
MAP amplitude (mmHg)	Difference of baseline MAP to nadir
SVR amplitude (mmHg.min ⁻¹ L ⁻¹)	Difference of baseline SVR to nadir
HR amplitude (bpm.min ⁻¹)	Difference HR baseline from HR peak
CO amplitude (L.min ⁻¹)	Difference CO baseline from CO peak
SV amplitude (mL)	Difference SV baseline from SV peak
Phase 1 (30s)	
SBP and DBP recovery amplitude (mmHg)	Highest SBP & DBP within 30 seconds after nadir
MAP recovery amplitude (mmHg)	Highest MAP within 30 seconds after nadir
SVR recovery amplitude (mmHg.min ⁻¹ L ⁻¹)	Highest SVR within 30 seconds after nadir
HR recovery peak (bpm.min ⁻¹)	Highest HR within 30 seconds after peak
CO recovery peak (L.min ⁻¹)	Highest CO within 30 seconds after peak
SV recovery peak (mL)	Highest SV within 30 seconds after peak
CO-SVR matching	Correlation between CO and SVR within 30s after nadir
Phase 2 (30-60s)	
SBP and DBP recovery amplitude (mmHg)	Highest SBP & DBP within 30 - 60 seconds after nadir
MAP recovery amplitude (mmHg)	Highest MAP within 30 - 60 seconds after nadir
SVR recovery amplitude (mmHg.min ⁻¹ L ⁻¹)	Highest SVR within 30 - 60 seconds after nadir
HR recovery peak (bpm.min ⁻¹)	Highest HR within 30 - 60 seconds after peak
CO recovery peak (L.min ⁻¹)	Highest CO within 30 - 60 seconds after peak

SV recovery peak (mL)	Highest SV within 30 - 60 seconds after peak
CO-SVR matching	Correlation between CO and SVR within 30-60s after nadir
Phase 3 (60-180s)	
SBP and DBP recovery amplitude (mmHg)	Highest SBP & DBP within 60 - 180 seconds after nadir
MAP recovery amplitude (mmHg)	Highest MAP within 60 - 180 seconds after nadir
SVR recovery amplitude (mmHg.min ⁻¹ L ⁻¹)	Highest SVR within 60 - 180 seconds after nadir
HR recovery peak (beats.min ⁻¹)	Highest HR within 60 - 180 seconds after peak
CO recovery peak (L.min ⁻¹)	Highest CO within 60 - 180 seconds after peak
SV recovery peak (mL)	Highest SV within 60 - 180 seconds after peak
CO-SVR matching	Correlation between CO and SVR within 60-180s after nadir
Phase 4 (300-420s)	
SBP and DBP recovery amplitude (mmHg)	Highest SBP & DBP within 300 - 420 seconds after nadir
MAP recovery amplitude (mmHg)	Highest MAP within 300 - 420 seconds after nadir
SVR recovery amplitude (mmHg.min ⁻¹ L ⁻¹)	Highest SVR within 300 - 420 seconds after nadir
HR recovery peak (bpm.min ⁻¹)	Highest HR within 300 - 420 seconds after peak
CO recovery peak (L.min ⁻¹)	Highest CO within 300 - 420 seconds after peak
SV recovery peak (mL)	Highest SV within 300 - 420 seconds after peak
CO-SVR matching	Correlation between CO and SVR within 300-420s after nadir

SBP: systolic blood pressure. DBP: diastolic blood pressure. MAP: mean arterial pressure. SVR: systemic vascular resistance. HR: heart rate. CO: cardiac output. SV: stroke volume.

CHAPTER 4: SYSTEMATIC REVIEW

Preface to the Manuscript written exclusively by Faith Olarinde

This systematic review was conducted as part of my proposed master's thesis examining cardiovascular, autonomic and respiratory responses during orthostatic stress testing in individuals with long-COVID. It was conceptualized in response to emerging clinical and scientific reports on persistent orthostatic intolerance and autonomic dysfunction in this population. This review, therefore, aimed to synthesize evidence on the use of active standing test (AST) to characterize cardiovascular, autonomic, and respiratory responses in individuals with long-COVID. To our knowledge, no prior work has synthesized evidence on physiological responses to the use of AST in long-COVID compared to healthy controls.

This study was reported in accordance with PRISMA guidelines and was pre-registered with PROSPERO (CRD-42024615872). The decision to focus on AST, a non-invasive and widely used orthostatic stress protocol, was informed by its diagnostic relevance in conditions such as postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension (OH), as well as the protocol's similarity to daily activities. The findings from this review contribute to a foundational understanding of how cardiovascular and autonomic responses are altered in individuals with long-COVID and help to inform future research in this area.

Contribution of Authors

Dr. Villar and I conceptualized the study and identified the methodology. I completed study's registration, articles search and screening, data extraction and quality of bias assessment. Albená Nunes de Silva contributed as a second reviewer for the article screening and quality of bias assessment. I drafted the original manuscript with primary revisions from Dr. Villar. All authors contributed academic expertise and to reviewing and editing the manuscript. I also acquired the Ruth Asper Scholarship in Kinesiology and Recreation Management and the Canadian Institutes of Health Research Tri-Council Agency Graduate Students Scholarship for this project.

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Using active standing orthostatic stress tests to assess physiological responses in individuals with long-COVID: A systematic review

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Abstract

Background: Individuals with long-COVID frequently report symptoms of orthostatic intolerance, such as light-headedness and fatigue upon standing, which may be linked to autonomic and cardiovascular dysfunction. The active standing test provides a simple, clinically relevant means to assess these impairments. This systematic review aimed to determine whether autonomic, cardiovascular, and respiratory responses are altered during active standing in people with long-COVID. **Methods:** A systematic search, reported according to PRISMA guidelines, was conducted in PubMed, MEDLINE, EMBASE, CINAHL, and Scopus for articles published between 2020 – 2025. Studies were included if they used the active standing test, involved adults (≥ 18 years), included a long-COVID and healthy control group, used continuous beat-by-beat measurements, and reported physiological outcomes. Risk of bias was assessed using the nine-point Newcastle-Ottawa Scale. The review was registered with PROSPERO (CRD42024615872). **Results:** Three studies (216 long-COVID and 186 healthy controls participants) met our inclusion criteria. Across all studies, individuals with long-COVID consistently exhibited elevated heart rate (HR) in both supine and standing positions. However, blood pressure findings were more variable, with one study reporting that 13% of participants met orthostatic hypotension criteria, while another reported significant increases in diastolic BP over time during standing. Long-COVID groups also showed reduced heart rate variability and impaired baroreflex sensitivity during standing. **Conclusion:** Individuals with long-COVID show consistent elevations in HR and impaired autonomic function during active standing, with subgroup-specific blood pressure abnormalities. These findings suggest altered cardiovascular-autonomic integration, and the paucity of literature emphasizes the need for research evaluating hemodynamic responses in individuals with long-COVID.

Keywords: long-COVID; orthostatic stress tests; active standing test; physiological responses; orthostatic hypotension

Introduction

Long-COVID, also referred to as the post-acute sequelae of SARS-CoV-2 infection (PASC), post-COVID-19 syndrome, or chronic COVID syndrome, is a multi-system condition defined by new, persistent, or worsening symptoms lasting for ≥ 3 months following a positive COVID-19 polymerase chain reaction (PCR) or rapid test kit test (National Academies of Sciences et al., 2024; Soriano et al., 2022). The Delphi consensus criteria and National Academies of Sciences, Engineering and Medicine consensus statement specify that these symptoms cannot be explained by alternative diagnoses (National Academies of Sciences et al., 2024; Soriano et al., 2022). Frequently reported symptoms include dizziness upon standing, fatigue, dyspnea, and “brain fog”, which significantly reduces the health-related quality of life and daily functioning of individuals with long-COVID (Davis et al., 2023; Sanchez-Ramirez et al., 2021; Statistics Canada, 2022). A systematic review found that nearly 38% of 4118 participants reported chronic fatigue and over half experienced measurable decline in quality of life 3 to 6 months post-infection, signifying the pervasive impact of this condition (Sanchez-Ramirez et al., 2021).

Orthostatic intolerance (OI), defined as a reduced ability to sustain upright posture due to insufficient neuro-cardiovascular compensation to gravitational shifts in blood distribution following a change in posture (Ricci et al., 2015), has also been reported to be prevalent in individuals with long-COVID (Blitshteyn & Whitelaw, 2021; Ormiston et al., 2022; Vernon et al., 2022). OI symptoms such as postural orthostatic tachycardia syndrome (POTS) and orthostatic hypotension (OH), contribute to debilitating symptoms like light-headedness and dizziness, which in turn lead to an increased risk of fainting, falls and injuries (Blitshteyn & Whitelaw, 2021; Monaghan et al., 2022; Ormiston et al., 2022; Vernon et al., 2022). POTS is

characterized by an increase in heart rate (HR) of ≥ 30 beats per minute (bpm) within 10 minutes of standing without a concurrent drop in blood pressure, while OH is traditionally defined as a decrease of ≥ 20 mmHg in systolic blood pressure (SBP) or ≥ 10 mmHg in diastolic blood pressure (DBP) within the first three minutes of standing (Freeman et al., 2011).

Evidence suggests that long-COVID is associated with dysfunctions across multiple physiological systems (Davis et al., 2023; Diab et al., 2021; Sanchez-Ramirez et al., 2021). Dysfunctions in normal cardiovascular responses to orthostatic stress tests have been reported in individuals with long-COVID (Bielecka et al., 2023; van Campen et al., 2024). Particularly, altered autonomic regulation, reflected by reduced baroreflex sensitivity and sympathetic-parasympathetic tone imbalance, and disrupted respiratory dynamics, which interact closely with cardiovascular control and venous return through perturbations in breathing patterns, may contribute to inadequate hemodynamic compensation (Davis et al., 2023; DePace & Colombo, 2022).

The active standing orthostatic stress test (AST), similar to the physiological demands of everyday activities, is a valuable tool for assessing orthostatic responses in clinical and research settings (Finucane et al., 2019). Studies also indicate that the average adult performs about 45 – 60 postural transitions in a day (Dall & Kerr, 2010; Finucane et al., 2019). Unlike the head-up tilt test (HUTT), which is a passive form of orthostatic stress testing involving passive tilting without skeletal muscle activation, AST involves muscle contractions compressing capacitance vessels in the lower leg (O’Connell et al., 2015; Ricci et al., 2015; Wieling et al., 2022) that support venous return, better reflecting the physiological mechanisms involved in day-to-day postural transitions (Wieling et al., 2022; Wieling & Groothuis, 2012). The effect of gravity upon standing induces a tirade of physiological responses, such as a reduced venous return and

activation of baroreceptors, increasing sympathetic outflow and decreasing parasympathetic tone, providing a window into cardiovascular and autonomic health (Finucane et al., 2019; Freeman et al., 2011). This distinction from the HUTT is critical, as the absence of active muscle contractions and the time it takes to move individuals to the upright tilt-table angle during HUTT may limit the ability to detect initial orthostatic hypotension (McJunkin et al., 2015; Pickering et al., 2005; Wieling et al., 2007), a transient but clinically significant drop in blood pressure of at least 40 mmHg in SBP and/or a decrease of at least 20 mmHg in DBP, occurring within the first 30 seconds of standing (Freeman et al., 2011; Moya et al., 2009; Ricci et al., 2015).

Advancements in beat-by-beat data collection and analysis have permitted further development of OH classifications, including initial orthostatic hypotension (IOH) and delayed orthostatic hypotension (DOH) based on the timing of the blood pressure drop (Freeman et al., 2011).

However, despite the advantages of the AST, significant variability still exists in the reports from AST research and clinical practice, leading to controversies in reported prevalence rates of POTS and OH in long-COVID populations. For example, Monaghan et al.'s (2022) study on orthostatic intolerance (OI) among older adults with long-COVID showed that only 13% (7 participants out of 53) exhibited an HR increase of >30 bpm upon standing. Intriguingly, among this subgroup, only 2% (1 participant) reported awareness of the heightened HR and accompanying sensations of light-headedness. In contrast, Blitshteyn et al.'s (2021) study reported a substantially higher prevalence of POTS, with 75% of participants ($n = 20$) exhibiting POTS. This variability is further exaggerated when the HUTT is used, as observed in González-Hermosillo's (2023) study, which showed no significant differences in HR but instead an exaggerated increase in blood pressure (González-Hermosillo et al., 2023). While these results may be due to limitations of existing studies such as differences in participant characteristics

(e.g., age, comorbidities), timing of assessment post-infection, small sample size, or general health of individuals prior to SARS-CoV-2 infection (Ferreira et al., 2024; Osuchowski et al., 2021), differences in test protocols could also be a reason, and there is an increasing importance to acknowledge the existing heterogeneity in the evidence, and recognize the limitations of specific studies, where measured and unmeasured factors may confound result interpretation.

Therefore, this systematic review aims to i) identify studies using the active-standing orthostatic stress test to characterize cardiovascular, autonomic, or respiratory responses in individuals with long-COVID, given its relevance to everyday activities and its diagnostic role for conditions such as POTS, OH, and OI; ii) evaluate the specific physiological variables measured, with an emphasis on cardiovascular, autonomic, and respiratory outcomes; and iii) assess methodological consistency across studies and explore how variations in active-standing protocols may contribute to heterogeneity in findings and their interpretation.

Methods

This systematic review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. The study's protocol was registered in the PROSPERO International database (CRD-42024615872). The Population, Exposure, Comparison, Outcome (PECO) framework was used to guide the research question and search strategy (Morgan et al., 2018). Using this framework, we defined the following: (P) Population: adults aged 18 years and older with long-COVID; (E) Exposure: active standing orthostatic stress test; (C) Comparison: responses in individuals with long-COVID compared to healthy controls; (O) Outcomes: quantitative autonomic, cardiovascular, and respiratory responses to AST. Therefore, our research questions were: 1) What is the current state of evidence on the use of active standing orthostatic stress test to assess cardiovascular, autonomic,

and respiratory responses in individuals with long-COVID? 2) What methodological limitations or gaps exist in this emerging body of literature?

Search strategy and selection criteria

Published literature indexed in PubMed, MEDLINE, EMBASE, CINAHL & Scopus from January 2020 to August 2024 was initially searched by FO in August 2024, and an updated search was performed in May 2025 using the University of Manitoba Libraries platform, with assistance from an experienced librarian (JW). Combinations of the following terms were used: long -covid', OR 'post covid' OR 'post-acute sequelae of SARS-CoV-2' or 'long-haul covid', 'orthostatic stress test', 'orthostatic hypotension', 'postural transition', 'hemodynamic responses', 'blood pressure', 'heart rate', 'cardiovascular', 'autonomic' and 'respiratory'. The search was limited to studies published in English language and from years 2020 – 2025. A sample search strategy for PubMed is presented in supplemental Appendix Supplemental 4.1.

Title and abstract screening criteria were independently screened by two review authors (FO and AN) using Covidence (<https://www.covidence.org>; Melbourne, Australia) following training and calibration on a random sample of abstracts to make sure both reviewers were on the same page and screening criteria was clarified where necessary by discussion between the lead (FO) and supervising (RV) author. Articles identified as potentially eligible by the two review authors were retrieved, and duplicates were either manually (when identified) or automatically (by Covidence) removed. Full texts of potentially eligible studies were screened independently by FO and AN, and RV resolved conflicts when necessary.

Studies were included if they: (1) reported on cardiovascular, autonomic or respiratory responses during the AST test in adults (≥ 18 years old) with long-COVID; (2) published between 2020 – 2025; (3) used continuous beat-by-beat measurements of blood pressure or HR; (4)

published in the English language. Studies were excluded if they did not use an active standing test, only used manual measurements of BP and HR, focused on assessing immunophenotypical biomarkers of long-COVID, used only self-reported surveys or questionnaires (DePaul symptom questionnaire, COMPASS-32, etc.), were published as systematic, scoping, narrative reviews, case studies, qualitative studies, poster or conference abstracts, letters to editors, or editorials.

Data extraction

Data extraction was independently performed by FO and AN. A standardized Excel template was used to extract relevant information pertaining to reference data (author, title, journal, year, and country), study characteristics (objective, design, testing setting), participant characteristics (age, sex, race, number of participants, and health status prior to COVID-19 infection). Study details, including the inclusion and exclusion criteria, proportion of participants who were hospitalized (severity of COVID-19 infection), current medication use, and reported symptoms, were also extracted. The instrumented (devices, active standing protocol, questionnaires), outcome measures (cardiovascular, autonomic, and respiratory variables), the definition of long-COVID used, conclusions and limitations as identified by the study authors, and funding sources were also extracted (Table 4.1). No automation tools were used in this process.

Collate, summarize and report results

Data from each article were extracted and entered into a standardized Excel template, with descriptive statistics reported for all variables related to the orthostatic stress test. If a study included multiple assessments (e.g. an active standing test (AST), a questionnaire, and Valsalva maneuver), all were noted; however, only quantitative variables related the AST were recorded in the Excel file. The variables extracted are presented in Table 4.1 below.

Table 4.1. *Variables extracted at full-text level.*

Reference information	Author(s)
	Title
	Journal
	Year
	Country
Study information	Study objective
	Study design
	Testing setting(s)
	Number of participants
	Inclusion and exclusion criteria
Demographic	Age
	Sex assigned at birth
	Race
	Symptoms reported
	Health status
	Medication use
	Hospitalization status during COVID-19
	infection

Instrumented measurement information	Techniques/devices used/instruments (protocol, questionnaires) Active standing protocol (timeframe)
Blood pressure and heart rate measurements	Continuous (e.g. using Finometer Finapres)
Outcome measures	Autonomic nervous system variables (Heart rate variability, etc).
	Cardiovascular system variables (Blood pressure, heart rate, cardiac output, etc.)
	Respiratory system variables (spirometry, volume of oxygen consumption and carbon dioxide production, etc.)
Final reports/Other information	Definition of long-COVID used (if applicable)
	Conclusions
	Limitations Funding sources/disclosures

Quality and Risk of Bias Assessment

Two independent reviewers (FO and AN) evaluated the quality and risk of bias for each included study using the nine-point Newcastle–Ottawa Quality Assessment Scale (NOS) for case-control studies (Wells et al., 2000). Disagreements were resolved by the supervising author (RV). The NOS was tailored to long-COVID studies. Studies were categorized based on the NOS scored as follows: 0–3 points were considered low quality, 4–6 points moderate quality,

and 7–9 points as high quality (Wells et al., 2000) across the categories of (i) selection, (ii) comparability, and (iii) exposure.

Since the NOS was originally designed for cohort and case-control studies, we adapted the case-control scoring tool for long-COVID observational cross-sectional studies by modifying some of its domains to better assess exposure and outcome, as described in previous work in the healthcare field (Herzog et al., 2013; Modesti et al., 2016). Specifically, we interpreted the domain ‘representative of long-COVID cases’ as meeting recognized definitions of long-COVID and if the sample recruited from relevant populations (e.g., community, outpatient clinics, tertiary care units, or post-COVID recovery programs), and interpreted ‘exposure’ as encompassing the process by which a study confirmed COVID-19 infection in its participants and whether the statistical test used was appropriate, fully described or incomplete. A meta-analysis could not be performed due to the small number of studies and heterogeneity across the studies. The modified scoring criteria is provided in Table 4.2 below.

Table 4.2. *Modified scoring of the Newcastle-Ottawa scale for long-COVID studies.*

Category	Original scoring criteria	Modified scoring criteria for long-COVID
Selection (maximum 4 points)	Is the case definition adequate? (maximum 1 point)	Did they state their definition of long-COVID? • Yes (1 point) • No, only stated definition of COVID-19 (0 points) • No definition provided (0 points)
	Representativeness of the cases. (maximum 1 point)	Is the sample representative of long-COVID cases? • Yes (1 point) • No (0 points)
	Selection of controls (maximum 1 point)	Is there inclusion of a healthy control group? • Healthy control (1 point) • No control group (0 points)
	Definition of controls (maximum 1 point)	Did they provide characteristics of the control group? • Yes (1 point) • No description of control group characteristics (0 points)

Comparability (maximum 2 points)	Comparability of cases and controls, controlling for confounding variables (maximum 2 points)	• The study controlled for the confirmation of long-COVID (the amount of time passed since infection and presentation for symptoms) (1 point) • The study controlled for age and sex of long-COVID to control subjects (age- and sex- matching) (1 point) • Study did not control for any of these factors (0 points)
Exposure (maximum 3 points)	Ascertainment of exposure (maximum 1 point)	How did they confirm COVID-19 infection? • Secure record (positive PCR or rapid kit test) (1 point) • Self-reported a probable infection with current long-COVID symptoms (1 point) • No confirmation or description (0 points)
	Same method of ascertainment for cases and controls (maximum 1 point)	Were the same diagnostic criteria used in determining if they had long-COVID used for the cases and the controls? • Yes (1 point)

	• No or not reported (0 points)
Non-response rate (maximum 1 point)	Was the statistical test appropriate and absolute outcome measures values fully reported? • Yes (1 point) • No or incompletely described (0 points)

Data synthesis and analysis

Descriptive characteristics were used to describe trials and participants. Means, medians, and associated measures of variability (e.g., 95% confidence intervals (CIs), interquartile ranges (IQRs)) were applied to continuous variables, while counts and proportions were used for dichotomous and categorical variables. Given the novelty of long-COVID, the anticipated heterogeneity in study designs, and the scarcity of data related to the research question, conducting a meta-analysis was not feasible. Descriptions of individual studies were extracted and presented to show methodological similarities and differences. A narrative summary of results was used to summarize and interpret findings.

Results

Study selection and search strategy

In total, 2145 references were identified and imported into Covidence for screening through database searches, with 1189 references removed as duplicates. 956 studies were screened by title and abstract following duplicate removal, and 41 studies were screened on the

full-text level. Three studies were included in the review following full-text screening. The flowchart of study selection is provided in Figure 4.1.

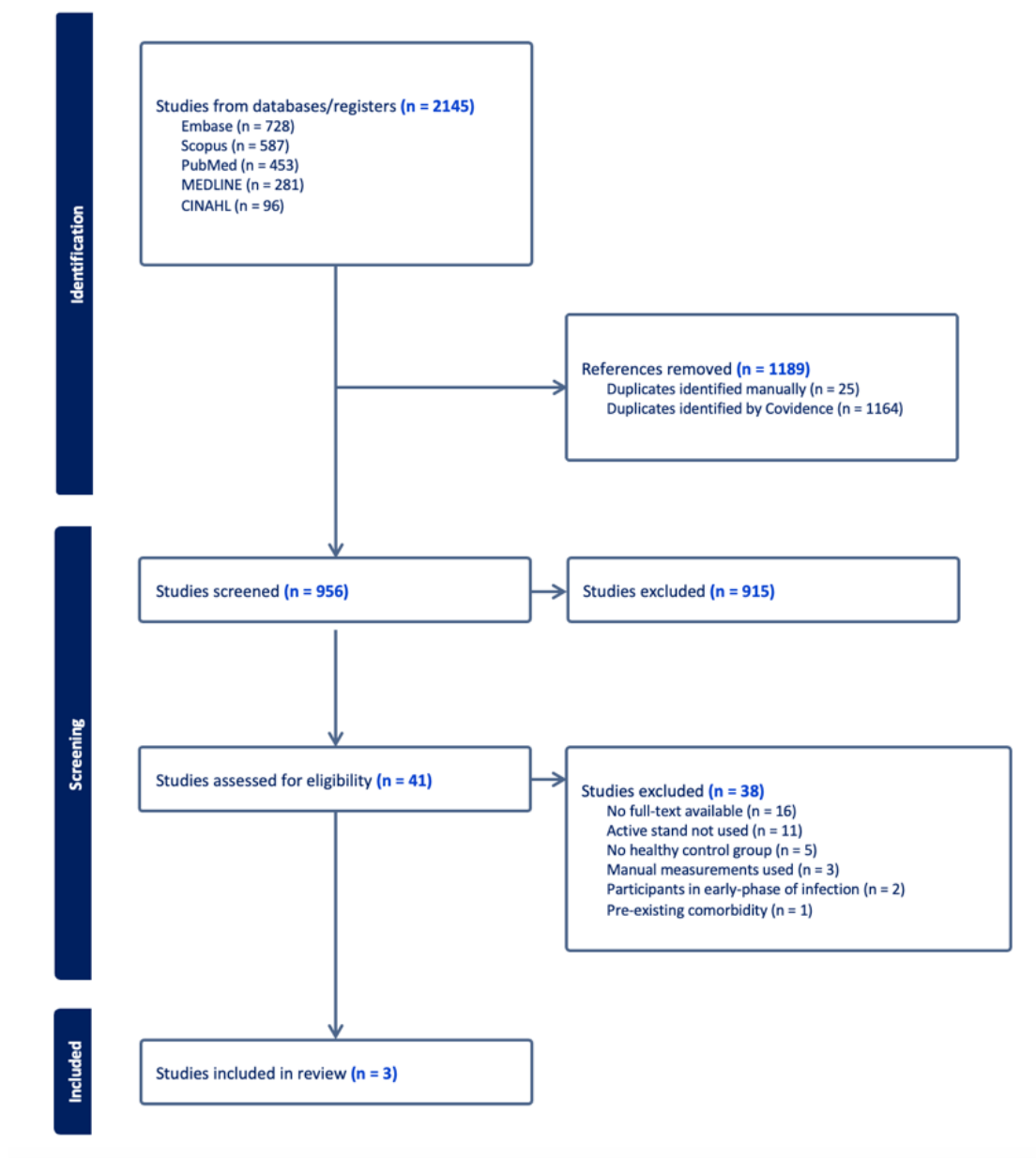


Figure 4.1: PRISMA flow chart. Identification and screening of studies

Study characteristics

We identified 3 studies that utilized the AST to evaluate physiological responses in individuals with long-COVID (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022). These

studies assessed a range of cardiovascular (e.g., heart rate, blood pressure), autonomic (e.g., heart rate variability, baroreflex sensitivity) and cardiorespiratory (respiratory sinus arrhythmia) variables. Each study included a healthy control group for comparison. Sample sizes varied across studies, ranging from 30-92 long-COVID participants and 33-120 healthy control participants. Methodological approaches differed in terms of data collection protocols (5 mins supine and 3 mins standing *vs.* 10 mins supine and 10 mins standing), and definition of long-COVID used (WHO's definition *vs.* NICE). The studies also differed in design, comprising one prospective single-center study, one prospective comparative study, and one cross-sectional study. Study classification was based on the descriptions provided by the original authors. However, despite these differences, all studies aimed to characterize orthostatic responses and identify potential impairments in autonomic or cardiovascular regulation in the long-COVID population. Study characteristics are presented in Table 4.3.

Table 4.3. *Study characteristics.*

Study (Year)	Country	Study design	Sample size	Proportio n of female (%)	Age (mean $\pm$ SD, median [IQR])/[CI] (years)	Definition of long-COVID	Active standing protocol (Timeframe)	Outcome Measures
Shah et al. (2022)	India	Prospective single center	PASC = 92; HC = 120	41% 46%	PASC = 50.6 ± 12.1 HC = 51.8 ± 4.2	Descriptive definition; no organizational source	5 min. supine, 3 min. stand	Heart rate variability
Seeley et al. (2023)	Australia	Prospective comparative	PASC = 30; POTS = 33 HC = 33	82% 94% 82%	PASC = 37 [15] POTS = 28 [14] HC = 28 [23]	WHO's Delphi consensus	10 min. supine, 10 min. standing	Respiratory sinus arrythmia; Heart rate and blood pressure responses

Hira et al. (2025)	Canada	Cross- sectional	PASC = 94; HC = 33	81% 76%	PASC = 42 [36, 53]; HC = 49 [30, 62]	NICE guideline	Heart rate variability,
							10 min. Blood pressure
							supine, 10 variability, Baroreflex min. sensitivity, Blood standing pressure and heart rate responses

SD: standard deviation; *IQR*: inter-quartile range; *CI*: confidence interval; *PASC*: post-acute sequelae of SARS-CoV-2 infection;

HC: healthy control; *OH*: orthostatic hypotension; *POTS*: postural orthostatic tachycardia syndrome; *WHO*: World Health

Organization; *NICE*: National Institutes for Health and Care Excellence.

Quality of bias assessment

Risk of bias was assessed using the nine-point Newcastle-Ottawa Quality Assessment Scale (NOS) adapted for cross-sectional long-COVID studies. All studies were classified as high quality (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022). The quality of included studies is summarized in Table 4.4.

Table 4.4. Risk of bias quality assessment.

	Shah et al., (2022)	Seeley et al., (2023)	Hira et al., (2025)
Case definition provided	1	1	1
Representativeness of long-COVID cases	1	1	1
Selection of controls	1	1	1
Definition of controls	1	1	1
Comparability of cases – control for time passed since symptom presentation	0	1	1
Comparability of cases – age- & sex- matching	1	0	1
Ascertainment of the exposure	1	1	1
Same method of ascertainment	1	1	1

Statistical test/absolute values reported	0	0	1
Total score	7	7	9
Quality	High	High	High

Score 0-3: low quality; 4-6: moderate quality; 7-9: high quality.

Definitions and Diagnostic Criteria

All three studies defined postural orthostatic tachycardia syndrome (POTS) as an increase in HR of ≥ 30 bpm within 10 minutes of standing, in the absence of OH (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022). OH was defined in Seeley et al. (2023) and Shah et al. (2022) as a ≥ 20 mmHg drop in SBP or ≥ 10 mmHg drop in DBP within 3 minutes of standing. Hira et al. (2025) instead only evaluated initial orthostatic hypotension (IOH), defined as a transient SBP drop of ≥ 40 mmHg within 15 seconds of standing, with recovery within 45–60 seconds. DBP was not considered in this definition.

Heart rate responses

All three studies (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022) reported elevated HR in long-COVID participants compared to healthy controls in both supine and upright positions. Supine HR was significantly higher in long-COVID groups, with reported averages ranging from 67 to 88 bpm, compared to 61 to 78 bpm in controls (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022). The increase in HR following standing was also significantly higher in long-COVID participants, particularly in those meeting the criteria for postural orthostatic tachycardia syndrome (POTS), with standing HR ranging from 99 to 114 bpm compared to 78–83 bpm in controls. Subgroup analysis comparisons in Hira et al. (2025) showed significant differences in supine and standing HR across long-COVID phenotypes, with the LC-POTS

group showing the highest increase after standing. Detailed HR responses across studies and subgroups are presented in Table 4.5.

Table 4.5. Comparison of heart rate findings across studies.

Study (Year)	Supine HR Findings	Standing HR Findings
Shah et al. (2022)	LC: 88 ± 15 bpm	LC-OH: 99 ± 18 bpm
	Controls: 78 ± 11 bpm	No-OH: 86 ± 14 bpm
	$p = 0.0001$.	$p = 0.006$
		Controls: not reported.
Seeley et al. (2023)	PASC: 72 ± 13 bpm	Δ HR (0-10 min):
	POTS: 79 ± 12 bpm	PASC: 36 [30–47] bpm
	Controls: 68 ± 9 bpm	POTS: 46 [34–59] bpm
	Group $p = 0.003$	Controls: 15 [9–20] bpm
	PASC > Control $p = 0.054$	Group $p < 0.001$
	PASC < POTS p : not reported	PASC > Control $p < 0.001$
		POTS > PASC $p = 0.10$
Hira et al. (2025)	LC: 67 [62–75] bpm > Controls:	LC: 89 [77–106] bpm
	61 [56–70] bpm	Controls: 78 [69–87] bpm
	$p = 0.010$	$p = 0.001$.
	<i>Subgroup:</i>	<i>Subgroup:</i>
	LC-IOH: 67 [61–77] bpm	LC-IOH: 85 [77–93] bpm
	LC-POTS: 68 [65–75] bpm	LC-POTS: 114 [103–131] bpm
	LC-none: 63 [57–73] bpm	LC-none: 80 [70–88] bpm

Controls: 61 [56–70] bpm	Controls: 78 [69–87] bpm
Group $p = 0.023$	Group $p < 0.001$
Subgroup p -values:	Subgroup p -values:
LC-none vs. controls ($p = 0.999$)	LC-none vs. controls ($p = 0.999$)
LC-none vs. LC-POTS ($p = 0.283$)	LC-none < LC-POTS ($p = 0.001$)
LC-none vs. LC-IOH ($p = 0.063$)	LC-none vs. LC- IOH ($p = 0.279$)
LC-POTS vs. LC-IOH ($p = 0.999$)	LC-POTS > LC-IOH ($p = 0.001$)

Long-COVID terminology presented as reported in each article. *OH*: orthostatic hypotension; *LC*: Long-COVID; *LC-OH*: Long-COVID with orthostatic hypotension; *PASC*: post-acute sequelae of COVID-19; *POTS*: postural orthostatic tachycardia syndrome; *HR*: heart rate; *LC-IOH*: participants with long-COVID and initial orthostatic hypotension; *LC-POTS*: participants with long-COVID and postural orthostatic tachycardia syndrome; *LC-none*: participants with long-COVID but no abnormalities.

Blood pressure responses

Blood pressure findings were less consistent across the included studies. Shah et al. (2022) reported that 13% of long-COVID participants ($n = 12/92$) met the criteria for orthostatic hypotension based on manually measured blood pressure, though absolute systolic (SBP) and diastolic (DBP) values and p -values were not reported for these variables (Shah et al., 2022). Similarly, Seeley et al. (2023) found no significant differences during supine and standing between groups ($p = 0.12$) but observed a time-dependent increase in DBP in both the PASC and POTS groups during standing compared to controls ($p = 0.03$), however, absolute values were not reported. In contrast, Hira et al. (2025) reported absolute BP values and found no significant SBP, DBP, or MAP differences between the long-COVID and control groups during supine and

standing. However, subgroup analysis showed that during supine, LC participants who met the criteria of IOH (LC-IOH) had significantly higher SBP, DBP, and MAP when compared to both LC-none and LC-POTS groups (all $p < 0.001$). After standing, group-level differences remained significant for SBP and MAP but not in DBP, with LC-IOH showing lower BP responses than LC-none and LC-POTS.

Additionally, vascular sympathetic modulation, measured by low-frequency systolic blood pressure variability (LF_{SBP}) was found to be significantly reduced in LC-none compared to controls during both supine and standing positions. Standing LF_{SBP} was also significantly lower in the LC-none group compared to the LC-POTS group. Detailed blood pressure responses across studies and subgroups are presented in Table 4.6 below.

Table 4.6. Comparison of blood pressure responses findings across studies.

Variable		Shah et al. (2022)	Seeley et al. (2023)	Hira et al. (2025)
SBP-supine (mmHg)	Absolute values	not reported.	No significant group differences ($p = 0.12$). No absolute values reported.	LC: 119 [109–132] mmHg Controls: 119 [114–124] mmHg $p = 0.683$.
				<i>Subgroup:</i> LC-IOH: 130 [123–143] mmHg LC-none: 113 [105–119] mmHg LC-POTS (112 [103–123] mmHg LC-IOH > LC-none ($p = 0.001$). LC-IOH > LC-POTS ($p = 0.001$).

DBP-supine (mmHg)	Absolute values not reported.	No data reported for DBP in supine.	LC: 71 [64–77] mmHg Controls: 67 [64–72] mmHg $p = 0.065$. <i>Subgroup:</i> LC-IOH: 76 [67–79] mmHg LC-none: 67 [63–73] mmHg LC-POTS: 66 [63–73] mmHg LC-IOH > LC-none ($p = 0.001$). LC-IOH > LC-POTS ($p = 0.009$).
MAP-supine (mmHg)	Not reported.	Not reported.	LC: 87 [80–95] mmHg Controls: 85 [80–91] mmHg $p = 0.268$. <i>Subgroup:</i> LC-IOH: 94 [87–100] mmHg LC-none: 81 [78–89] mmHg LC-POTS: 81 [77–92] mmHg LC-IOH > LC-none ($p = 0.001$). LC-IOH > LC-POTS ($p = 0.001$).
LF _{SBP} supine (mmHg ²)	Not reported.	Not reported.	LC: 4.06 [2.69–6.62] mmHg² Controls: 5.15 [3.77–9.37] mmHg² $p = 0.074$. LC-none < controls ($p = 0.001$).

SBP-standing (mmHg)	Absolute values not reported. OH identified in 13% of participants.	No significant differences (group*time interaction); $p = 0.14$).	LC: 113 [99–128] mmHg Controls: 115 [107–132] mmHg $p = 0.241$. Group main effect: $p = 0.001$. <i>Subgroup:</i> LC-IOH: 124 [112–136] mmHg LC-none: 109 [95–126] mmHg LC-POTS: 102 [96–117] mmHg LC-IOH > LC-none ($p = 0.031$) LC-IOH > LC-POTS ($p = 0.001$)
DBP-standing (mmHg)	Absolute values not reported.	DBP increased over time in PASC and POTS vs. controls (Group $p = 0.001$). POTS > controls ($p < 0.03$); PASC > controls $p < 0.03$ PASC vs. POTS ($p = 0.74$).	LC: 72 [64–79] mmHg Controls: 71 [66–76] mmHg $p = 0.999$. Group main effect not significant ($p = 0.135$).
			LC: 87 [75–96] mmHg Controls: 86 [81–97] mmHg

MAP-Standing (mmHg)	Not reported.	Not reported.	$p = 0.534$. Group main effect ($p = 0.007$). <i>Subgroup:</i> LC-IOH: 90 [84-100] mmHg LC-none: 84 [72-91] mmHg LC-POTS: 79 [74-92] mmHg LC-IOH > LC-none ($p = 0.049$) LC-IOH > LC-POTS ($p = 0.019$)
LF _{SBP} -Standing (mmHg ²)	Not reported.	Not reported.	LC: 0.5 [6.19-14.8] mmHg² Controls: 14.5 [9.05-30] mmHg² $p = 0.001$. <i>Subgroup:</i> Controls: 14.5 [9.05-30] mmHg² LC-none: 6.59 [4.22-12] mmHg² LC-POTS: 10.2 [4.49-17] mmHg² LC-none < controls ($p = 0.001$) LC-none < LC-POTS ($p = 0.042$)

SBP: Systolic blood pressure; *DBP*: Diastolic blood pressure; *MAP*: Mean arterial pressure;

LF_{SBP}: Low-frequency systolic blood pressure; *LC-IOH*: participants with long-COVID and initial orthostatic hypotension; *LC-POTS*: participants with long-COVID and postural orthostatic tachycardia syndrome; *LC-none*: participants with long-COVID but no abnormalities.

Time-domain heart rate variability

Both Shah et al. (2022) and Hira et al. (2025) reported decreases in heart rate variability (HRV) in individuals with long-COVID compared to healthy controls (Hira et al., 2025; Shah et al., 2022). Time-domain HRV indices, such as root-mean square of successive R-R interval differences (RMSSD) and standard deviation of R-R intervals (SDNN), were consistently lower in long-COVID participants compared to healthy controls. Shah et al. (2022) also reported a reduction in RMSSD based on COVID-19 severity, finding that RMSSD values were lowest in those with severe and moderate illness, with higher values in the mild and asymptomatic groups ($p < 0.0001$). Similarly, subgroup analysis by Hira et al. (2025) also showed significant reductions in HRV in LC-IOH and LC-POTS subgroups compared to LC-none group during standing. Standing RMSSD was lowest in LC-POTS group (9.72 [7.37-13] ms) and highest in LC-none group (19 [16-38] ms), with similar patterns observed for SDNN and percentage of successive R-R intervals differing by more than 50 ms (pNN50). Detailed time-domain HRV across studies and subgroups are presented in Table 4.7 below.

Table 4.7. Time Domain HRV indices.

Variable	(Shah et al., 2022)	(Hira et al., 2025)
RMSSD (ms)	LC: 13.9 ± 11.8 ms Controls: 19.9 ± 19.5 ms	<i>Supine:</i> No significant group-level differences.
	LC < Controls ($p = 0.01$)	<i>Standing:</i>
	<i>Subgroup:</i>	LC: 15 [8.9–22] ms
	Graded reduction by COVID	Controls: 18 [13–32] ms
	severity ($p = 0.0001$).	LC < Controls ($p = 0.011$).

	Asymptomatic: 24.2 ms Mild: 16.3 ms Moderate: 9.3 ms Severe: 7.2 ms	<i>Subgroup:</i> LC-IOH: 15 [9.60–27] ms LC-none: 19 [16–38] ms LC-POTS: 9.72 [7.37–13] ms LC-POTS < LC-none ($p = 0.001$) LC-POTS < LC-IOH ($p = 0.024$)
SDNN (ms)	LC: 16.9 ± 12.9 ms Controls: 22.5 ± 17.6 ms LC < Controls ($p = 0.01$).	<i>Supine:</i> No significant group-level differences. <i>Standing:</i> LC: 31 [22–43] ms Controls: 40 [32–55] ms; LC < Controls ($p = 0.001$). <i>Subgroup:</i> LC-none: 37 [28–51] ms LC-POTS: 27 [20–33] ms LC-POTS < LC-none ($p = 0.018$)
pNN50 (%)	Not reported.	<i>Supine:</i> No significant group-level differences. <i>Standing:</i> LC: 0.23 [0–0.87] ms Controls: 0.78 [0.11–2.72] ms Long-COVID < Controls ($p = 0.011$).

Subgroup:

LC-IOH: 2.46 [0.32–7.44]%

LC-none: 0.83 [0–2.50]%

LC-POTS: 8.12 [3.10–13]%

LC-POTS > LC-none ($p = 0.039$)LC-POTS > LC-IOH ($p = 0.026$)

LC: long-COVID; *HC*: healthy controls; *RMSSD*: root-mean square of successive R-R interval differences; *SDNN*: standard deviation of R-R intervals; *pNN50*: percentage of successive R-R intervals differing by more than 50ms. *LC-IOH*: participants with long-COVID and initial orthostatic hypotension; *LC-POTS*: participants with long-COVID and postural orthostatic tachycardia syndrome; *LC-none*: participants with long-COVID but no abnormalities.

Frequency-domain heart rate variability

Frequency-domain HRV indices, including high-frequency HRV (HF_{HR}), low-frequency HRV (LF_{HR}), total power HRV (TP_{HR}), LF/HF ratio and vagal baroreflex sensitivity ($BRSv$), were reported only by Hira et al. (2025). No significant differences were found between long-COVID and control participants during supine. However, after standing, long-COVID participants demonstrated significantly lower HF_{HR} , LF_{HR} , and TP_{HR} compared to controls, with no group differences found in LF/HF ratio or $BRSv$. Subgroup analysis revealed significant differences in HF_{HR} , LF_{HR} , TP_{HR} , and $BRSv$ across long-COVID phenotypes (LC-IOH, LC-POTS, LC-none) in both supine and standing positions. During supine, the LC-IOH group showed significantly lower HF_{HR} , LF_{HR} , and $BRSv$ compared to LC-POTS (all $p < 0.05$), and $BRSv$ was also significantly reduced in LC-IOH vs. LC-none ($p = .010$). Following standing, the LC-POTS group had significantly lower HF_{HR} , LF_{HR} , and TP_{HR} compared to LC-none (all $p <$

0.01). BRSv was also significantly lower in both LC-POTS and LC-IOH compared to LC-none. LF/HF ratio did not significantly differ between groups. Detailed frequency-domain HRV across studies and subgroups are presented in Table 4.8. below.

Table 4.8. Summary of findings of frequency variables from Hira et al. 2025 study.

Variable	Supine Findings	Standing Findings
HF_{HR}	No significant difference between LC and controls.	LC: 315 [95–730] ms ² Controls: 349 [260–1190] ms ² LC < Controls ($p = 0.042$).
	<i>Subgroup:</i>	<i>Subgroup:</i>
	Control: 1136 [262-3121] ms ²	Control: 349 [260-1190] ms ²
	LC-none 1919 [725-4921] ms ²	LC-none 578 [337-1881] ms ²
	LC-IOH: 1384 [339-2036]ms ²	LC-IOH: 296 [98-871]ms ²
	LC-POTS: 2166 [1112-4419] ms ²	LC-POTS: 100 [51-339] ms ²
	Group $p = 0.026$ ms ²	Group $p < 0.001$ ms ²
	LC-POTS > LC-IOH ($p = 0.028$).	LC-none > LC-POTS ($p = 0.001$).
LF_{HR}	No significant differences between LC and controls.	LC: 1203 [483–2212] ms ² Controls: 1901 [935–4008] ms ² LC < Controls ($p = 0.008$).
	<i>Subgroup:</i>	<i>Subgroup:</i>
	Control:1484 [603-4210] ms ²	Control:1901 [935-4008] ms ²
	LC-none: 2130 [1142-4393] ms ²	LC-none: 1806 [1106-3932] ms ²
	LC-IOH: 1429 [643-2778] ms ²	LC-IOH: 974 [428-1941] ms ²
	LC-POTS: 2680 [1889-4938] ms ²	

TP_{HR}	Group $p = 0.015 \text{ ms}^2$	LC-POTS: 1080 [298-1671] ms^2
	LC-POTS > LC-IOH ($p = 0.012$).	Group $p = 0.001 \text{ ms}^2$
		LC-POTS < LC-none ($p = 0.020$)
	No significant differences between LC and controls.	LC: 3313 [1773–5559] ms^2
		Controls: 5342 [3410–10550] ms^2
		LC < Controls ($p < 0.001$)
	<i>Subgroup:</i>	<i>Subgroup:</i>
	Control: 689 [2547-17839] ms^2	Control: 5342 [3410–10550] ms^2
	LC-none 7702 [4681-14480] ms^2	LC-none: 4938 [301-9406] ms^2
	LC-IOH: 5975 [3321-11206] ms^2	LC-IOH: 3269 [1757-5130] ms^2
BRS_v	LC-POTS: 9415 [5096-16398] ms^2	LC-POTS: 2619 [1413-3949] ms^2
	Group $p = 0.057 \text{ ms}^2$	Group $p < 0.001 \text{ ms}^2$
	LC-POTS > LC-IOH ($p = 0.030$).	LC-POTS < LC-none ($p = 0.006$).
	No significant differences between LC and controls.	No significant differences between LC and controls.
	<i>Subgroup:</i>	<i>Subgroup:</i>
	Controls: 6.89 [4.94-11.4] ms/mmHg	Controls: 3.97 [2.93-6.41] ms/mmHg
	LC-none: 10.7 [5.35-17.0] ms/mmHg	LC-none: 5.33 [3.34-7.06] ms/mmHg
	LC-IOH: 6.24 [4.11-10.0] ms/mmHg	LC-IOH: 2.77 [1.76-5.11] ms/mmHg
	LC-POTS: 9.81 [8.47-16.0] ms/mmHg	LC-POTS: 2.85 [1.44-3.93] ms/mmHg
	Group $p < 0.001$	Group $p < 0.001$

	LC-POTS > LC-IOH ($p = 0.010$)	LC-POTS < LC-none ($p < 0.001$)
	LC-IOH > LC-none ($p = 0.001$).	LC-IOH < LC-none ($p < 0.005$)
LF/HF Ratio	No significant differences between groups or subgroups.	No significant differences between groups or subgroups.

HF_{HR} : high-frequency HRV; LF_{HR} : low-frequency HRV; TP_{HR} total power HRV. BRS_v vagal baroreflex sensitivity. *LC-IOH*: participants with long-COVID and initial orthostatic hypotension; *LC-POTS*: participants with long-COVID and postural orthostatic tachycardia syndrome; *LC-none*: participants with long-COVID but no abnormalities.

Respiratory sinus arrhythmia

Only Seeley et al. (2023) reported that respiratory sinus arrhythmia, which was significantly lower in their PASC group (9.8 (7.0 – 14.5) breaths/min) compared to the POTS (14.0 (12.1 – 19.2) breaths/min) and healthy control (17.4 (12.0 – 19.5) breaths/min) groups ($p < 0.001$).

Discussion

This systematic review aimed to i) identify studies using the active-standing orthostatic stress test to characterize cardiovascular, autonomic, or respiratory responses in individuals with long-COVID compared to healthy controls; ii) evaluate the specific physiological variables measured; and iii) assess methodological consistency across studies to explore how potential sources of heterogeneity in findings. Across the included studies, individuals with long-COVID demonstrated increased heart rate and reduced heart rate variability, while blood pressure responses varied across long-COVID subgroups.

Only three studies to date have used the active standing test (AST) to characterize physiological responses in individuals with long-COVID compared to healthy controls (Hira et

al., 2025; Seeley et al., 2023; Shah et al., 2022). Across these studies, heart rate (HR) and blood pressure (BP) were the most commonly reported outcomes, with consistent evidence of elevated HR responses during standing in the long-COVID participants compared to healthy controls. Although definitions of POTS and OH were largely consistent across studies, the extent and pattern of physiological responses varied within long-COVID subgroups (Hira et al., 2025; Seeley et al., 2023; Shah et al., 2022). The identification of distinct subgroups within the long-COVID population (LC-IOH, LC-POTS, LC-none etc.) reinforces the diagnostic value of AST for detecting autonomic dysfunction in long-COVID. These findings suggest that HR responses may serve as a sensitive early marker of dysautonomia in individuals suspected of having long-COVID (Aydin et al., 2017; Uppal et al., 2025).

Cardiovascular indices are commonly assessed during orthostatic stress testing and are central to diagnosing conditions such as POTS and OH (Low et al., 2013; Plash et al., 2013). In the studies reviewed, HR and BP measurements were the most frequently reported variables. While these are valuable markers of autonomic function, the broader impact of long-COVID on multiple physiological systems, including cardiovascular, cerebrovascular, musculoskeletal, autonomic, respiratory, endocrine systems is increasingly evident but still needs to be clarified (Evcik, 2023; Hira et al., 2023; Novak et al., 2022).

Despite this complexity of autonomic and cardiovascular regulation, research to date has primarily focused on overt measures such as BP and HR, as they are easier to measure and analyze. However, other key hemodynamic variables such as cardiac output, systemic vascular resistance, cerebral blood flow, and baroreflex sensitivity remain underreported, limiting a more integrated understanding of physiological function in long-COVID (Khan et al., 2025; Smith et al., 1994; Tanaka et al., 1996). A lack of attention to compensatory mechanisms during

orthostatic stress tests that may mask autonomic and cardiovascular dysfunction (e.g., altered vascular resistance) also limits the ability to fully characterize the extent of hemodynamic instability in this population.

In addition, methodological differences across studies using the AST, including variation in the duration of supine and standing phases (e.g., 5 minutes supine and 3 minutes standing in Shah et al. (2022) *vs.* 10 minutes supine and 10 minutes standing in Seeley et al. (2023) and Hira et al. (2025) further complicates interpretation. The use of different measurement tools (manual BP *vs.* continuous beat-by-beat monitoring) and the omission of absolute BP and HR values, also limit cross-study comparisons. Incomplete data reporting continues to pose interpretative challenges and emphasizes the need for more comprehensive and standardized physiological reporting in future long-COVID studies.

Additionally, inconsistencies in how long-COVID is defined across studies contribute to heterogeneity. Seeley et al. (2023) used the World Health Organization's (WHO) "post-COVID condition" definition, which defines it as the development of new or persistent symptoms occurring at least three months after the initial SARS-CoV-2 infection, lasting for a minimum of two months and not attributable to alternative diagnoses (Soriano et al., 2022). In contrast, Shah et al. (2022) cited the National Institutes of Health (NIH) definition, which is less specific, broadly describing long-COVID as health issues that persist for months after infection without a defined timeframe (National Institutes of Health, 2024). Also, none of the included studies adopted the 2024 consensus criteria proposed by the National Academies of Sciences, Engineering, and Medicine (NASEM), describing long-COVID as an "infection-associated chronic condition" that arises after SARS-CoV-2 infection, lasts at least three months, and presents with continuous, relapsing-remitting, or progressive symptoms affecting one or more

organ systems, emphasizing the chronic and multi-systemic nature of long-COVID and aims to standardize classification for research and clinical purposes (National Academies of Sciences et al., 2024). This definitional variability complicates cross-study comparisons and may introduce differences in the classification of symptom severity, long-COVID duration, and phenotype classification within long-COVID cohorts. As research in this area advances, adoption of standardized case definition, such as the one proposed by NASEM, will be essential to improve comparability of findings across studies.

Proposed physiological mechanisms

Dysautonomia, a dysfunction in the autonomic nervous system that includes conditions like POTS, neurocardiogenic syncope, and OI, poses a significant physiological challenge as it requires a coordinated neuro-cardiovascular response to maintain adequate BP, HR, and organ perfusion (Fedorowski et al., 2023; Novak et al., 2022; O’Connell et al., 2015; Ricci et al., 2015). OI is characterized by a reduced ability to sustain an upright posture and often manifests as OH occurs when the cardiovascular system fails to compensate for reduced venous return upon standing (Ricci et al., 2015). Although OI is often associated with frailty in older adults, recent research has shown that people experiencing long-COVID also experience this condition. It leads to symptoms such as dizziness, light-headedness and faintness, increasing the risk of syncope, falls, and related injuries like fractures and head trauma (Monaghan et al., 2022; Ormiston et al., 2022; Vernon et al., 2022).

While evidence suggests autonomic dysfunction in people experiencing long-COVID, studies vary in how they define and assess it. BP and HR measurements are the most commonly used metrics, as seen in all three studies included in this systematic review. However, these responses still vary across studies. For instance, Seeley et al. (2023) found that 79% of their

long-COVID sample ($n = 33$) met the criteria for POTS during the active standing orthostatic stress test. In contrast, Shah et al. (2022) found that only 2% of their sample ($n = 92$) met the POTS criteria. Similarly, a study by Monaghan et al. (2022) reported that none of their long-COVID sample ($n = 85$) met POTS criteria during active standing. Interestingly, when the same participants underwent the head-up tilt test, 13% of the 85 met the POTS criteria. However, despite 13% of participants meeting the POTS criteria, only 2% (1 participant) reported awareness of the increased HR and accompanying sensations of light-headedness (Monaghan et al., 2022).

In addition to autonomic dysfunction, cardiovascular responses observed during active standing, while variable, may also point to altered hemodynamic control in long-COVID. While Seeley et al. (2023) found a rise in DBP after standing, without a significant difference found in SBP between their long-COVID, POTS and control groups, Hira et al. (2025), in contrast, found a significant difference in SBP and MAP in their groups. These alterations in responses indicate potentially impaired cardiovascular compensations, which may be due to blunted baroreflex activity, reduced venous return, stroke volume or systematic vascular resistance, although these variables were not measured across the identified studies (Chapleau, 2012; Creager & Creager, 1994; Hira et al., 2025; Izar et al., 2012). The lower low-frequency SBP variability (LF_{SBP}) in long-COVID groups also indicates disrupted vascular sympathetic modulation (Hira et al., 2025; Parati et al., 1995, 2018).

The pathophysiology of cardio-autonomic dysfunctions in individuals with long-COVID remains speculative, as dysautonomia can arise from various factors, including viral invasion of autonomic centers or hypothalamic impairment, which could either be neuronal or hematogenous (Davis et al., 2023; Serviente et al., 2022). However, the exaggerated inflammatory response (or

cytokine storm) triggered by SARS-CoV-2 infection is often cited as the primary mechanism underlying dysautonomia in individuals with long-COVID (Acosta-Ampudia et al., 2022; Chen et al., 2024; Rojas et al., 2022). In relation to this inflammatory response, some studies have shown that individuals with long-COVID tend to show increased sympathetic activity at rest compared to individuals without long-COVID, although some others have reported parasympathetic hyperactivity as measured by HRV (Ferreira et al., 2024; Marques et al., 2023; Shah et al., 2022; Zacher et al., 2023). However, it is suspected that COVID-19 affects the sympathovagal balance of the autonomic nervous system, and the variability in results that exists in the literature might be attributed to factors such as the timing of post-infection autonomic control assessments, age of study participants, disease severity, and general health of individuals prior to SARS-CoV-2 infection (Ferreira et al., 2024; Osuchowski et al., 2021).

Recommendations for future research

The existing literature on long-COVID and the use of active standing orthostatic stress test (AST) in evaluating autonomic, cardiovascular, and respiratory responses primarily focuses on HR and BP as key cardiovascular parameters. However, a more comprehensive assessment, including underreported hemodynamic measures such as stroke volume, cardiac output, systemic vascular resistance, and the matching between cardiac output and systemic vascular resistance, could provide a better understanding of autonomic and cardiovascular dysfunction (De Matos et al., 2025). Using standardized long-COVID definitions, diagnostic criteria, and orthostatic stress testing protocols could also improve comparability across studies. Greater emphasis on continuous beat-by-beat monitoring, instead of intermittent manual measurements, may also help detect transient abnormalities that are often overlooked.

The inclusion of well-matched healthy control groups and comparative analyses with other post-viral syndromes such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), could also aid in clarifying whether autonomic dysfunction observed in long-COVID is specific to the condition or part of a broader post-viral sequelae recovery process. Additionally, due to the unexpected emergence of long-COVID from the COVID-19 pandemic, pre-post-test designs are not often feasible. Therefore, the use of healthy control groups, ideally individuals without chronic conditions that could potentially confound the results (e.g., hypertension), should be used as a comparison. Alternatively, comparing individuals with long-COVID with those from established post-viral syndromes like ME/CFS, may help improve interpretation of autonomic and cardiovascular abnormalities (Komaroff & Lipkin, 2023).

Due to the multi-system nature of COVID-19, integrating multi-system assessments such as the cerebrovascular, respiratory, and cardiovascular responses within AST protocols could also provide a more comprehensive understanding of long-COVID's impact on the human physiology. This approach would be particularly useful for longitudinal studies assessing the trajectory of physiological responses over time and distinguishing transient dysfunction phases from persistent dysautonomia and cardiovascular dysregulation. Furthermore, given the discrepancies in incidence rates and symptom reporting, future research should continue to explore the role of demographic factors, sex differences, infection severity, and pre-existing health factors in the long-COVID population. This could improve risk stratification and also guide the development of targeted therapeutic strategies to mitigate long-term impairment in affected individuals.

Strengths and limitations

To the best of our knowledge, this is the first systematic review that synthesizes the current literature on long-COVID and its effects on autonomic, cardiovascular, and respiratory responses to active standing orthostatic stress. The AST was chosen as the primary focus due to its similarity to activities of daily living, making it a relevant tool for understanding how individuals with long-COVID respond to postural changes in a real-world context. Our inclusion criteria was designed to include studies that specifically used the active standing protocol while also ensuring a high degree of methodological rigor. Studies were required to include both a long-COVID group and a control group, as this allowed us to directly compare the physiological responses between individuals with long-COVID and healthy individuals or other relevant groups. We also limited our selection to studies published between 2020 and 2025, to ensure the review reflects all of the most current understanding of long-COVID research.

To ensure consistent and accurate physiological data, we included only studies that employed continuous measurements of key variables such as BP and HR. This is particularly important for capturing transient abnormalities in autonomic and cardiovascular responses that may be missed with intermittent measurements (e.g., manual BP or automated BP). We also required studies to assess physiological variables related to the autonomic, cardiovascular, and respiratory systems. These variables are essential for evaluating physiological regulation and understanding how dysfunctions may manifest in individuals experiencing long-COVID. However, only one study assessed a cardiorespiratory variable.

Despite these rigorous criteria, we found that the number of eligible studies was relatively small, which prevented us from conducting a meta-analysis. Moreover, the variables assessed across the included studies were not as comprehensive as we had hoped, limiting the depth of

analysis we could perform on BP regulation and other autonomic functions and cardiovascular short-term responses in this population. This emphasizes the need for more studies that incorporate a broader range of physiological variables and employ more detailed measurement techniques (e.g. beat-by-beat photoplethysmography).

Conclusion

This systematic review identified and synthesized studies using the active standing test to assess cardiovascular, autonomic, and respiratory responses in individuals with long-COVID compared to healthy controls. While limited in number, the included studies showed significant increases in heart rate responses and reduced heart rate variability in individuals with long-COVID, especially within subgroups exhibiting POTS and initial orthostatic hypotension, suggesting persistent autonomic dysfunction. Blood pressure responses were more variable across studies, with some subgroup level differences in systolic and mean arterial pressure, though these were not consistently observed across studies. This review also identified the absence of standardized long-COVID definitions, missing reports of key data (absolute BP and HR values), and the absence of other hemodynamic variables (e.g., cardiac output, stroke volume, systemic vascular resistance) and respiratory measures, which limited our ability to compare findings and continues to present a gap in understanding the patterns of responses of physiological dysfunction. Future research should consider adopting the use of standardized definitions (e.g., NASEM's consensus criteria), use continuous hemodynamic measurement tools, and include broader physiological assessing variables beyond BP and HR. Additionally, including healthy control groups or relevant post-viral comparison groups could be important in understanding long-COVID patterns of dysregulation. If possible, a multi-system assessment approach could also improve the characterization of physiological responses and may inform

targeted interventions to help individuals with long-COVID manage the persistent burden of the condition.

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Other information

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The authors of this paper declare no conflict of interest for this systematic review.

Author contribution

Faith Olarinde: Writing – original draft, Writing – review & editing, Methodology, Conceptualization. **Albena Nunes-Silva:** Methodology. **Diana Sanchez-Ramirez:** Formal review. **Yannick Molgat-Seon:** Formal review. **Rodrigo Villar:** Supervision, Conceptualization, Methodology, Validation, Formal analysis. All authors provided critical review for important intellectual content and approved the final version of the manuscript.

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CHAPTER 5: CROSS-SECTIONAL STUDY

Preface to the Manuscript written exclusively by Faith Olarinde

This cross-sectional study was conducted as originally proposed during my master's thesis proposal, with some modification to the analyzed variables due to time constraints and examines cardiovascular responses during orthostatic stress testing in individuals with long-COVID. It was conceptualized based on my interest in reports of autonomic dysfunction in this population, as well as the gaps identified through the systematic review I conducted (and presented in Chapter 4). This study therefore was designed to address some of the gaps and aimed to assess a comprehensive set of cardiovascular variables during an active standing test (AST), compare responses between individuals with and without long-COVID, evaluate the frequency of occurrence of orthostatic hypotension, assess symptom burden using the DePaul Symptom Questionnaire, and explore short-term compensatory mechanisms. The study was initially designed to include age- and sex- matched long-COVID and non-long-COVID groups, however, during analysis, it became clear that some participants in the control group met the criteria for orthostatic hypotension. After consulting with my advisor (Dr. Villar) and the committee members (Dr. Sanchez-Ramirez and Dr. Molgat-Seon), a decision was made to conduct a subgroup analysis, dividing participants into 3 groups. This adjustment impacted age and sex matching and led to an uneven sample size across groups. To my knowledge, no previous study have examined or compared this proposed range of cardiovascular variables during an active standing test in individuals with long-COVID compared to healthy controls, and also using a phased approach. This manuscript follows the STROBE reporting guidelines and received ethics approval from the University of Manitoba Research Ethics Board 1 (REB-1) (HE2024-0364).

Contribution of Authors

Dr. Villar and I conceptualized the study and identified the methodology with Dihogo De Matos and Jefferson Lima de Santana. I submitted the ethics application, recruited participants, trained research assistants, coordinated data collection, and analyzed the data (trained and supported by Dihogo De Matos and Jefferson Lima de Santana). I also drafted the original manuscript with primary revisions from Dr. Villar. All authors contributed academic expertise and to reviewing and editing the manuscript. I also received fundings from the Ruth Asper Scholarship in Kinesiology and Recreation Management and the Canadian Institute for Health Research Canada Graduate Scholarship (Masters) (CIHR CGS-M).

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Unveiling the unseen: Exploring orthostatic hypotension and intolerance symptoms in long-COVID

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Abstract

Background: Long-COVID (LC) is associated with dysfunction in the cardiovascular, respiratory, and autonomic systems, with persistent symptoms such as shortness of breath, fatigue, and orthostatic intolerance. These symptoms can significantly impair daily functioning and quality of life. However, the underlying mechanisms remain poorly understood. **Objective:** This study aims to report the frequency of occurrence of orthostatic hypotension (OH) and orthostatic intolerance (OI) symptoms in individuals with long-COVID, and to compare phase-specific cardiovascular responses to orthostatic stress between long-COVID participants and age- and sex-matched healthy controls. **Methods:** 13 LC participants and 13 NLC participants, aged 18+, completed a 5-minute sitting and a 10-minute supine followed by 7 minutes of standing active standing orthostatic stress test. Beat-by-beat blood pressure and heart rate were recorded using a Finometer and ECG. **Results:** OH was observed in 85% of LC participants ($n = 13$; 64% initial OH, 36% classical OH) and 69% of NLC participants ($n = 13$; 13% initial OH, 67% COH). Cardiovascular responses in NLC participants were sub-grouped into those with orthostatic hypotension (NLC_{OH}) and those with a normal blood pressure response (NLC_{NBPR}). Baseline SBP and HR did not differ significantly across groups (SBP: $p = 0.249$; HR: $p = 0.914$). However, SBP amplitude drop differed significantly between all groups ($p < 0.001$), including significantly lower in LC vs. NLC_{OH} ($p = 0.015$) and in NLC_{OH} vs. NLC_{NBPR} ($p < 0.001$) during the lie-to-stand protocol. DBP, SVR, MAP and CO were also found to be significantly different across groups. No significant differences were found in recovery HR or all assessed variables across phases 1-4. **Conclusion:** The high frequency of occurrence of IOH ($\geq 40/20$ mmHg BP drop) with minimal HR compensation in LC suggests autonomic cardiovascular dysfunction. Routine orthostatic testing may aid in early identification and characterization of post-COVID

cardiovascular impairments. As the first study to examine phase-specific cardiovascular trends in this population, these findings warrant replication in a larger sample.

Keywords: long-COVID; active standing orthostatic stress test; cardiovascular responses; orthostatic hypotension; blood pressure regulation; postural transition

Introduction

Long-COVID, a condition resulting from infection by the severe acute respiratory coronavirus (SARS-CoV-2) and characterized by persistent, relapsing-remitting, or progressive symptoms that affect one or more organ systems, lasting at least three months post-infection, has emerged as a debilitating health concern with substantial impacts on healthcare systems and the global economy. Although much attention has been given to its clinical symptoms, the underlying physiological mechanisms of long-COVID remain poorly understood, particularly regarding the regulation of the autonomic nervous system and cardiovascular function (Bliddal et al., 2021; Davis et al., 2023; Serviente et al., 2022). This gap in understanding poses a challenge to the potential development of effective therapeutic interventions for those suffering from the long-term effects of the virus. In Canada, approximately 3.5 million people are affected by long-COVID, with many reporting reduced functional capacity as a result of persistent symptoms, including fatigue, brain fog, light-headedness, and breathlessness (Arnold et al., 2021; Government of Canada, 2024; Kokolevich et al., 2022; Sanchez-Ramirez et al., 2021).

Among the most debilitating and commonly reported symptoms in individuals with long-COVID are those related to autonomic dysfunction such as dysregulated blood pressure and heart rate responses. These cardiovascular issues often manifest as orthostatic hypotension (OH) and postural tachycardia syndrome (POTS), where the body fails to properly regulate blood pressure and heart rate during orthostatic stress (DePace & Colombo, 2022; González-Hermosillo et al., 2023; Hira et al., 2025; Podoleanu et al., 2009; Vernon et al., 2022). However, despite growing recognition of these dysfunctions, current studies remain divided regarding specific cardiovascular dysfunctions that characterize long-COVID. While some studies report significantly reduced heart rate variability and blood pressure dysregulation, the findings remain

variable across studies (Hira et al., 2023; Monaghan et al., 2022; Seeley et al., 2023; Shah et al., 2022).

A potential reason for these inconsistent findings is the lack of detailed analysis of cardiovascular responses across different time phases following postural changes. Orthostatic challenges, such as transitioning from sitting or lying to standing are common daily activities performed by humans on an average of 45-60 times per day (Smith et al., 1994; Wieling & Groothuis, 2012b). These postural transitions require quick, dynamic, and coordinated responses from both the cardiovascular and autonomic systems (Smith et al., 1994; Wieling & Groothuis, 2012b). When these systems fail to compensate adequately for gravity-induced changes in blood pressure, individuals can experience symptoms such as light-headedness and dizziness, which are often reported in individuals with long-COVID (Dani et al., 2021; DePace & Colombo, 2022). However, despite the importance of these postural challenges, most studies have not explored how cardiovascular responses adjust over time during these transitions. As a result, the dynamic and delayed compensatory mechanisms of the cardiovascular system remain poorly understood in the long-COVID population, where these mechanisms may be impaired.

Orthostatic hypotension (OH), typically defined as a drop of at least 20 mmHg in systolic blood pressure (SBP) or at least 10 mmHg in diastolic blood pressure (DBP) within 3 minutes of assuming an upright position (Freeman et al., 2011), is one of the most common manifestations of cardio-autonomic dysfunction. While a drop in blood pressure is typical and expected upon standing due to the influence of gravity on the vascular system, orthostatic stabilization typically occurs within 1 minute in healthy individuals. (Ricci et al., 2015). However, in individuals with comorbidities bringing about dysautonomia, as is the case of long-COVID, different subtypes of orthostatic hypotension may occur, including 1) initial orthostatic hypotension (marked by a

decrease of at least 40 mmHg in SBP and/or a decrease of at least 20 mmHg in DBP within 30 seconds of standing); 2) classical orthostatic hypotension (a decrease in SBP of at least 20 mmHg or diastolic of at least 10 mmHg within 30 seconds to 3 minutes); and 3) delayed orthostatic hypotension (characterized by a gradual progressive drop in blood pressure of at least 20/10 mmHg or at least 15 mmHg in individuals with hypertension between 3 and 45 minutes) (Freeman et al., 2011; Moya et al., 2009; Ricci et al., 2015). While these subtypes have been identified in individuals with long-COVID (Hira et al., 2025; Monaghan et al., 2022), the majority of studies have focused on isolated time points of manual measurements, leaving a gap in understanding how cardiovascular responses adjust over time (Buoite Stella et al., 2022; Shah et al., 2022). There's also very limited research on how cardiovascular variables beyond heart rate (e.g., stroke volume, systemic vascular resistance, cardiac output) respond to postural changes during orthostatic challenges, therefore further limiting our understanding of compensation for orthostatic stress in individuals with long-COVID.

This study, therefore, aims to address these gaps by comprehensively analyzing cardiovascular responses specifically SBP, DBP, mean arterial pressure (MAP), systemic vascular resistance (SVR), heart rate (HR), cardiac output (CO), stroke volume (SV), and CO-SVR matching across four time phases: phase 1 (0-30 s), phase 2 (30-60 s), phase 3 (60-180 s), and phase 4 (300-420 s) during an active standing test. This phased approach is based on typical physiological recovery patterns in healthy adults and established orthostatic hypotension criteria, allowing for the assessment of adaptive responses and differences in cardiovascular regulation (Podoleanu et al., 2009; Romero-Ortuno et al., 2010).

The specific objectives are to: i) describe and compare the frequency of occurrence of orthostatic hypotension (OH) and intolerance symptoms; ii) assess and compare symptom

profiles using the DePaul Symptom Questionnaire – Short Form (DSQ-SF); and iii) compare cardiovascular responses across specific time phases of an active standing orthostatic stress test between individuals with and without long-COVID, focusing on identifying delayed or impaired compensatory responses. We hypothesize that the long-COVID group will report and show a higher frequency of occurrence for OH and OI symptoms, significant difference in DSQ-SF, and pronounced cardiovascular responses, including a greater drop in SBP and DBP, blunted increase in HR, reduced SVR, CO, SV, and a delayed CO-SVR matching compared to the control groups.

Methods

Study design

This observational cross-sectional study is reported in accordance with the Strengthening the Observational Studies in Epidemiology (STROBE) statement (Von Elm et al., 2007) and the Sex and Gender Equity in Research (SAGER) guidelines (Heidari et al., 2016) (See Appendix Supplemental 5.1).

Setting

This study took place in the Cardiorespiratory and Physiology of Exercise Research Laboratory in the Applied Research Centre at the University of Manitoba, Canada. Participant recruitment and data collection occurred between August 2024 to March 2025. Participants attended a single study visit for a duration of approximately 2.5 hours. The temperature of the lab space was controlled (~22-23°C) to avoid shivering, and humidity and barometric pressure were left ambient. Data collection appointment times was standardized to mornings, starting between 8:30-10 am, to avoid participants being influenced by diurnal variations in autonomic and cardiovascular function, such as fluctuations in blood pressure and heart rate that typically occur during the day (Faraci & Scheer, 2024; Shea et al., 2011).

Ethics

This study was approved by the Research Ethics Board 1 (REB-1), University of Manitoba Fort Gary Campus (HE2024-0364).

Participants

Individuals aged 18-80 years were recruited for this study through convenience sampling, which included flyers posted on social media and notice boards, referrals, word of mouth, and a post-COVID rehabilitation center. Interested individuals were contacted via phone and email to assess eligibility criteria based on the inclusion criteria. All eligible participants were also provided with pre-test instructions 48 hours before their scheduled test date, which they were advised to follow on the test day. The pretest instructions are presented in Appendix C.

The criteria for long-COVID followed the National Academies of Sciences, Engineering and Medicine consensus definition (National Academies of Sciences et al., 2024). Inclusion criteria for the long-COVID (LC) group are as follows: i) aged 18-80 years; ii) a probable or confirmed positive on a COVID-19 PCR or rapid antigen test; iii) experiencing current symptoms related to SAR-CoV-2 infection for at least 3 months prior to study appointment; iv) have independent mobility (i.e., mobile without the use of an assistive device). The non-long-COVID (NLC) group was age- and sex-matched to the long-COVID prior to conducting a subgroup analyses, with the NLC participants meeting the criteria of no current symptomology related to SARS-CoV-2 infection.

Exclusion criteria for both groups included pregnant individuals, those with pre-existing chronic health conditions affecting the cardiovascular, autonomic, or respiratory systems (e.g., hypertension, chronic obstructive pulmonary disease) prior to COVID-19, and individuals unable to abstain from medication use for 24 hours prior to the test.

Demographic, Physical Activity and Anthropometric Measurements

Demographic questions, including sex assigned at birth, date of birth, age, and information relating to SARS-CoV-2 infection (e.g., most recent date of infection, severity of infection, hospitalization status) were collected to characterize the sample. Anthropometric parameters were also measured, including height (Seca Deutschland, Hamburg, Germany), body mass (InBody Canada Co. Ltd., Ontario, Canada), and waist and hip circumference (tape measure). Whole body segmental bio-electrical impedance was also assessed with the InBody-270 body composition analyzer (InBody Canada Co. Ltd., Ontario, Canada), which measures an individual's total body water, dry lean mass, skeletal muscle mass, body fat mass, body mass index, lean muscle mass, and percentage of body fat. Self-reported physical activity was measured using the Paffenbarger Physical Activity Questionnaire (Tonstad et al., 2018), which measures weekly energy expenditure based on common physical activities. Medication use was also recorded (See appendix Supplemental 5.2), although participants were instructed to abstain from medications 24 hours prior to testing if applicable.

Study protocol

Participants arrived at the lab in a fasted state (having abstained from water or food for at least 2 hours prior to test time). Upon arrival, participants were provided with a consent form and provided with sufficient information to voluntarily consent to participation. They were also informed they could withdraw consent at any point. The 14-item DePaul Symptom Questionnaire (DSQ-SF) was then completed. Resting heart rate and blood pressure measurements were taken using an automated sphygmomanometer (Welch Allyn Flexiport™, Welch Allyn Inc., New York, USA), as well as oxygen saturation levels using a pulse oximeter (Ohmeda Biox 2700, Biox Technology Inc., Boulder, Colorado) to ensure participants values

were below cut-off (BP reading of $\leq 160/90$ mmHg, HR ≤ 100 bpm and SPO₂ $\geq 92\%$) (Canadian Society for Exercise Physiology, 2017).

Participants completed two active postural transition tests: (a) 5 minutes sitting to up to 7 minutes standing (SS) and (b) 10 minutes lying to up to 7 minutes standing (LS), representing a low and high orthostatic stress challenge, respectively. The order of the protocols was randomized and counterbalanced to control for potential order and carryover effects. A 10-minute seated break period was also provided in between conditions. Participants were also instructed to stand still during up to 7 minutes, minimizing any sways or movements (Finucane et al., 2019). After the test, participants were asked if they experienced any symptoms relating to orthostatic intolerance (e.g. light-headedness, dizziness) upon standing.

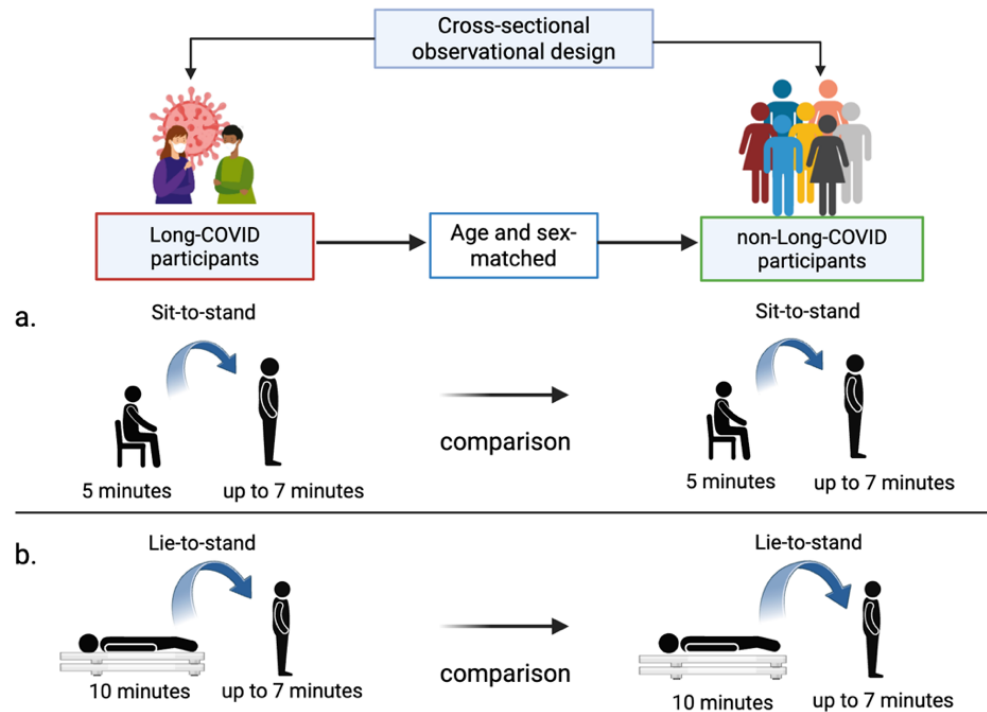


Figure 5.1: Illustration of the research design and experimental conditions (a and b).

Instrumentation and Data Sources

Cardiovascular variables of interest – systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), systematic vascular resistance (SVR), heart rate (HR), cardiac output (CO) and stroke volume (SV) – were continuously monitored using the finger cuff plethysmograph device (Finapres Medical System, Colorado Springs, CO, USA) and integrated into LabChart 8 software (ADInstruments). Beat-by-beat HR calculations were derived using a 3-lead ECG setup (electrodes placed on the right arm [RA], right leg [RL], and left leg [LL]), recorded through an Octal Bio amp module (ADInstruments, Finapres Medical System, Colorado Springs, CO, USA). R-waves were detected in LabChart 8 software, and inter-beat intervals were used to compute HR (in bpm) (LabChart 8, ADInstruments, Colorado Springs, CO, USA). Stroke volume (in mL) was derived from the formula: $SV = (CO / HR) \times 1000$. MAP was calculated as the average of the arterial pressure waveform over each cardiac cycle using LabChart's cyclic measurement function (LabChart 8, ADInstruments, CO, USA). SVR was estimated by dividing MAP by CO ($SVR = MAP / CO$). Cardiac output was calculated using the model flow algorithm within LabChart, with beat detection and finger pulse correction applied (ADInstruments, LabChart 8, Colorado Springs, CO, USA).

Orthostatic Hypotension and Postural Orthostatic Tachycardia Syndrome Classification

Orthostatic hypotension was defined as a drop of at least 20 mmHg in systolic blood pressure or at least 10 mmHg in diastolic blood pressure (DBP) within 3 minutes of assuming an upright position (Freeman et al., 2011). We also further classified this response into either initial orthostatic hypotension (IOH) (marked by a decrease of at least 40 mmHg in SBP and/or a decrease of at least 20 mmHg in DBP within 30 seconds of standing), classical orthostatic hypotension (COH) (a decrease in SBP of at least 20 mmHg or diastolic of at least 10 mmHg

within 30 seconds to 3 minutes), or delayed orthostatic hypotension (DOH) characterized by a gradual progressive drop in blood pressure of at least 20/10 mmHg or at least 15 mmHg in individuals with hypertension between 3 and 45 minutes (Freeman et al., 2011; Moya et al., 2009; Ricci et al., 2015). Postural orthostatic tachycardia syndrome (POTS) was characterized as an increase in heart rate of 30 bpm or more within 10 minutes of assuming an upright position, and this occurs without orthostatic hypotension (Freeman et al., 2011).

DePaul Symptom Questionnaire Short Form (DSQ-SF)

The 14-item self-reported DSQ-SF was used to assess symptomology related to myalgia encephalomyelitis/chronic fatigue syndrome (ME/CFS), a condition that shares clinical similarities with long-COVID, particularly in relation to post-exertional malaise (PEM), fatigue, cognitive dysfunction, among others (Buntić et al., 2024; Komaroff & Lipkin, 2023; Wold et al., 2024). Participants rated the severity and frequency of symptoms experienced over the past six months on a Likert scale. Frequency was rated as: 0 = none of the time, 1 = a little of the time, 2 = about half the time, 3 = most of the time and 4 = all of the time. Severity was rated as 0 = symptom not present, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe. The frequency and severity scores for each symptom were then averaged to create one score per symptom, and this average was multiplied by 25 to create the composite score out of 100 (Bedree et al., 2020). ME/CFS scoring was assessed using the Canadian Consensus Criteria case definition (Carruthers et al., 2003). Participants were considered to meet the criteria if they reported at least one symptom with frequency and severity ratings ≥ 2 in each of the following domains: fatigue, post-exertional malaise, sleep problems, and neurological/cognitive problems.

Study size

No sample size calculations were performed prior to the start of the study, as this is an exploratory study. The results obtained in this study will be used for sample size calculations in further studies.

Data processing and statistical analysis

Beat-by-beat cardiovascular data (SBP, DBP, MAP, SVR, HR, CO, SV) were processed in LabChart 8.0 (ADInstruments, LabChart 8, Colorado Springs, CO, USA) and MATLAB R2024 (MATLAB R2024, MathWorks, Inc., USA), time-aligned at zero, marking the transition point. Preprocessing involved removing motion artifacts using median and low pass filter (0.05 rad/sample) and applying a 5-second moving average filter to minimize the influence of inter-beat and inter-breath oscillations (van der Velde et al., 2007). Data were resampled at 1 Hz to correct for time delays, and baseline values were calculated from an average of the final 120 seconds before standing. Following transition, the lowest values for SBP, DBP, MAP, SVR and the highest values for HR, CO, and SV within the first 30 seconds after standing were used to assess initial cardiovascular response. Peak recovery points defined short-term compensatory responses were assessed across four time phases: phase 1 (0-30 s), phase 2 (30-60 s), phase 3 (60-180 s), and phase 4 (300-420 s). This phased approach is based on expected physiological responses in healthy adults and OH classifications.

We initially conducted a t-test to compare between long-COVID (LC) and non-long-COVID (NLC) groups. However, upon analysis, we found that some participants in the NLC group exhibited orthostatic hypotension (OH). As a result, participants in the NLC group were reclassified into subgroups based on their response during the lie-to-stand protocol. Overall, the groups included: LC, NLC with OH (NLC_{OH}), and NLC with a normal blood pressure response

(NLC_{NBPR}).

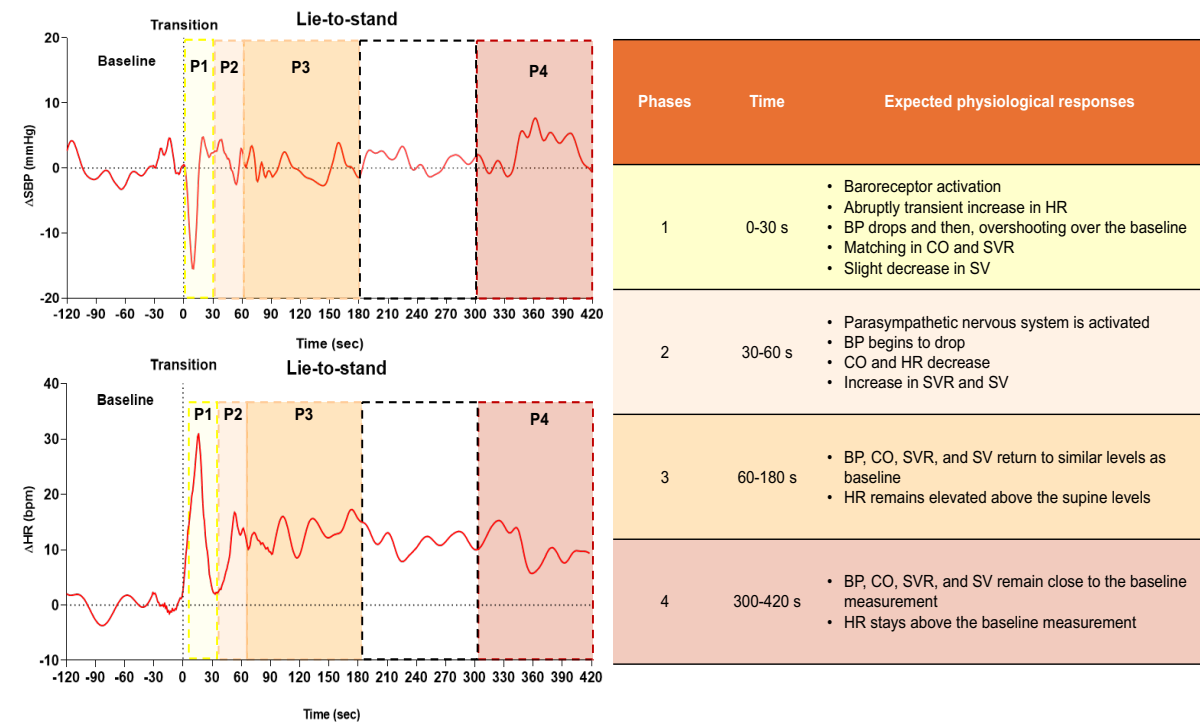


Figure 5.2: Example of systolic blood pressure (ΔSBP , top) and heart rate (ΔHR , bottom) responses to lie-to-stand divided into 4 phases.

Phase 1: 0-30 s, phase 2: 30-60 s, phase 3: 60-180 s, and phase 4: 300-420 s after transition.

*The white area (180-300 s) represents a stabilization phase similar to phase 3, and is unrelated to any OH classification, and therefore was not included in the analysis.

Statistical analyses were conducted in Jamovi v.2.6 (The Jamovi Project, Sydney, Australia), with data visualization in GraphPad Prism v.8 (Prism, GraphPad Software Inc., USA). Normality and heterogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. For normally distributed data, one-way ANOVA was used to compare across the LC, NLC_{OH}, and NLC_{NBPR} groups. The NLC group was subdivided based on participants' responses to the lie-to-stand protocol, which imposes a stronger orthostatic

challenge. Where homogeneity of variance was met (Levene's $p > 0.05$), *Tukey's HSD* was used for *post-hoc* comparisons. Where homogeneity of variance was violated (Levene's $p < 0.05$), *Games-Howell post-hoc* was used due to unequal sample size and variance (Parab & Bhalerao, 2010).

Non-normally distributed data were analyzed using the Kruskal-Wallis test, and the *Dwass-Steel-Critchlow-Fligner (DSCF) post-hoc* test was used when group differences reached statistically significance differences (Ranganathan, 2021). To correct for multiple hypothesis testing, the p -values were corrected using the *Benjamini-Hochberg* adjustment, with a false discovery rate (FDR) set at 0.05 (Benjamini & Hochberg, 1995). Parametric continuous data were reported as mean $\pm$ standard deviation (SD) with 95% confidence intervals (CIs), non-parametric variables were reported as median [interquartile range] (IQR) and minimum and maximum values, and categorical variables as frequencies and percentages. Fisher's exact tests were conducted to compare the proportion of participants with OH, IOH, and COH between the LC and NLC groups.

Effect sizes were determined using Cohen's d for parametric data, Cohen's r for non-parametric data, and partial eta squared (η^2) for ANOVA (Cohen, 1992; Lakens, 2013). Effect sizes was classified as small ($d = 0.2$; $\eta^2 \leq 0.05$), medium ($d = 0.5$; $\eta^2 = 0.06$), high ($d > 0.8$; $\eta^2 = 0.14$) (Cohen, 1992). Correlations between ΔCO and ΔSVR at phases 1 – 4 were analyzed using Pearson's (normal distribution) or Spearman's (non-parametric) correlation. Correlation strength was classified as very high (0.9-1.0), high (0.7-0.9), moderate (0.5-0.7), low (0.3-0.5), and negligible (0.0 – 0.3) (Aggarwal & Ranganathan, 2016; Mukaka, 2012). Statistical significance was set at $p < 0.05$ and adjusted for multiple comparisons when required.

Results

Participant characteristics

A total of 26 participants were included in this study ($n = 13$ LC, $n = 13$ NLC). Overall cohort analysis demonstrated that there were no statistically significant differences between all variables. The majority of participants were female (77%) and Caucasian (92%) in the LC group. Only 3 participants in the NLC group reported having tested positive for COVID-19. No participants in either group were hospitalized at any point during their infection, though three participants in the LC group reported seeking urgent care during their illness. After subdividing the NLC groups into NLC_{OH} and NLC_{NBPR} , due to meeting the criteria for orthostatic hypotension, body mass was the only variable found to be significantly different across groups (group $p = 0.009$; LC *vs.* NLC_{OH} $p = 0.013$; NLC_{OH} *vs.* NLC_{NBPR} $p = 0.015$). Participant characteristics are described in Table 5.1.

Table 5.1. *Participants characteristics- overall cohort and across subgroups.*

Overall cohort (<i>n</i> = 26)				NLC Subgroup analysis		LC, NLC _{OH} , & NLC _{NBPR}
Variables/groups	LC (<i>n</i> = 13)	NLC (<i>n</i> = 13)	<i>p</i> -value	NLC _{OH} (<i>n</i> = 9)	NLC _{NBPR} (<i>n</i> = 4)	<i>p</i> -value ^c
Age (years)	43.2 ± 16.7	41.6 ± 18.8	0.819	40.1 ± 20.9	45.0 ± 15.0	0.710
Sex, <i>n</i> (%)						
Males	3 (23%)	3 (23%)	–	3 (33%)	0	–
Sex, <i>n</i> (%)						
Females	10 (77%)	10 (77%)	–	6 (67%)	4 (100%)	–
Height (cm)	168.0 ± 8.3	167.7 ± 8.6	0.928	171.3 ± 6.4	159.8 ± 7.8	0.144
Body Mass (kg)	80.0 ± 16.4↑	69.1 ± 11.1	0.057	74.3 ± 9.0↑	57.4 ± 3.4	0.009*
BMI (kg/m ²)	28.5 ± 6.5	24.4 ± 3.0	0.055 ^a	25.3 ± 3.0	22.5 ± 1.9	0.097
Skeletal Muscle Mass (kg)	29.5 ± 6.3	27.7 ± 5.7	0.453	29.8 ± 5.2	23.1 ± 4.1	0.128
Body Fat Mass (kg)	27.1 ± 12.8	19.1 ± 7.31	0.064 ^a	20.9 ± 7.1	15.0 ± 6.9	0.152
PA Level (kcal/week)	5761.8 ± 3705.8 [§]	3936.9 ± 2555.7	0.145 ^b	3966.1 ± 2943.5	3871.3 ± 1736.3	0.321
Days since last infection	750.0 ± 464	785.0 ± 266	0.903	926.5 ± 147.79 [§]	503.0	0.654

Race, <i>n</i> (%)						
Caucasian	12 (92.3%)	5 (38.5%)	–	3 (33.3%)	2 (50.0%)	–
Asian	–	2 (15.4%)	–	2 (22.2%)	–	–
Latin American	1 (7.7%)	1 (7.7%)	–	–	1 (25.0%)	–
Black/African	–	3 (23.1%)	–	3 (33.3%)	–	–
Middle Eastern	–	1 (7.7%)	–	–	1 (25.0%)	–
Slavic	–	1 (7.7%)	–	1 (11.1%)	–	–

LC = Long-COVID; NLC = Non-Long-COVID. Values are presented as means (standard deviations) for continuous variables and counts (percentages) for categorical variables. ↑ statistically higher than. Statistical comparisons were conducted using Student's *t*, Mann-Whitney U test or Welch *t*-test as appropriate. Welch's *t*-test was used when the assumption of normality was met, but homogeneity of variances wasn't met. LC, NLC_{OH}, and NLC_{NBPR} *p*-value was conducted using a Kruskal-Wallis test. ^aWelch *t*-test, ^bMann-Whitney U-test. ^cKruskal-Wallis test. [§]PA level reported in 10 LC participants; [§]Days since last infection: only 3 NLC participants reported positive for COVID-19.

*Statistically significant differences between groups.

Participants in the LC group rated the severity of their illness as mild ($n = 8$), moderate ($n = 2$), and severe ($n = 3$), while the 3 participants in the NLC group reported their illness as mild, and the remaining participants ($n = 10$) reported no known infection of COVID-19 infection. Commonly reported symptoms in the LC group included fatigue (62%), brain fog (69%), unexplained shortness of breath after mild activity (46%), and inability to fully return to work due to persistent symptoms (69%). The flowchart for study recruitment is presented in Figure 5.3.

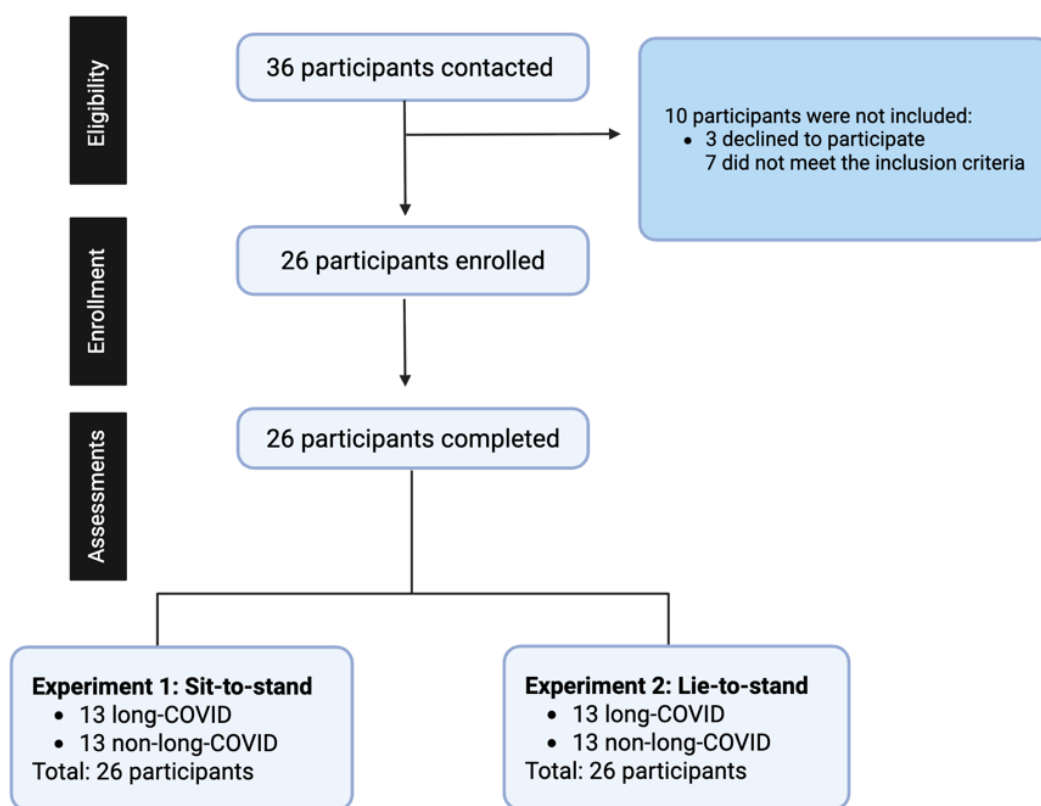


Figure 5.3: Flowchart study recruitment.

DePaul Symptom Questionnaire Short Form data

Out of the 14 DSQ-SF items, 11 (79%) was found to be significantly different between groups including fatigue, next day soreness, tiredness after minimum exercise, feeling unrefreshed after sleep, muscle pain or aches, problem remembering things, difficulty paying attention, unsteadiness after standing, cold limbs, unexplained hotness

or coldness and feelings of sickness from smells. There were no statistically significant differences between groups for bloating, irritable bowel problems, and flu-like symptoms. According to the Canadian Consensus Criteria case definition, only one participant in the LC group met the criteria for ME/CFS. Table 5.2 presents the results for the DSQ-SF symptomology scores in both the LC and NLC groups. The median (IQR) composite DSQ-SF scores are shown, along with the 95% confidence intervals (CIs), effect sizes (Cohen's r), and p -values for between-group comparisons. Data is missing for 3 LC and 2 NLC participants.

Table 5.2. *DePaul Symptom Questionnaire – Short Form (DSQ-SF).*

	LC Median [IQR] ($n = 10$)	NLC Median [IQR] ($n = 11$)	LC 95% CI ($n = 10$)	NLC 95% CI ($n = 11$)	p	Effect size
Fatigue/extreme tiredness	81.3 [46.88]↑	0.0 [25.00]	50.68 – 94.32	-1.52 – 31.06	<.001*	-0.85
Next day soreness or fatigue	50.0 [87.50]↑	0.0 [0.00]	19.60 – 80.40	-2.02 – 8.84	0.007*	-0.64
Tiredness after minimum exercise	50.0 [71.88]↑	0.0 [18.75]	26.67 – 85.83	-2.98 – 27.98	0.011*	-0.64
Feeling unrefreshed after sleep	68.6 [43.75]↑	25.0 [25.00]	43.34 – 84.16	5.62 – 37.56	0.003*	-0.75

Muscle pain or aches	50.0 [53.13]↑	0.0 [0.00]	16.23 – 63.77	-5.01 – 23.19	0.023*	-0.54
Bloating	50.0 [50.00]	25.0 [43.75]	28.03 – 75.72	7.79 – 42.21	0.068	-0.47
Problem remembering things	75.0 [50.00]↑	0.0 [6.25]	37.92 – 87.08	-1.12 – 10.21	<.001*	-0.85
Difficulty paying attention	75.0 [46.88]↑	0.0 [25.00]	34.11 – 88.39	1.33 – 28.22	0.008*	-0.67
Irritable bowel problems	0.0 [43.75]	0.0 [25.00]	-1.70 – 49.20	2.56 – 22.44	0.783	-0.07
Unsteadiness while standing	25.0 [40.63]↑	0.0 [0.00]	11.28 – 43.72	-2.79 – 7.34	0.005*	-0.65
Cold limbs	37.5 [50.00]↑	0.0 [0.00]	10.12 – 54.88	-2.25 – 11.34	0.026*	-0.51
Unexplained hot/coldness	37.5 [71.88]↑	0.0 [0.00]	11.47 – 68.53	-5.58 – 14.67	0.015*	-0.54
Flu-like symptoms	12.5 [43.75]	0.0 [0.00]	3.34 – 39.16	-3.37 – 17.01	0.130	-0.34
Feeling of sickness from smells	25.0 [50.00]↑	0.0 [0.00]	6.94 – 55.56	-3.02 – 14.38	0.039*	-0.47

Values are presented as median [IQR] as well as 95% confidence intervals (CIs). *p*-value between group differences provided as calculated using the Mann-Whitney U test. ↑ statistically higher than.

*Statistically significant differences compared to LC.

Frequency of occurrence of orthostatic hypotension, postural orthostatic tachycardia syndrome and orthostatic intolerance symptoms

Table 5.3 displays the frequency of occurrence of orthostatic hypotension and intolerance based on the lie-to-stand protocol. In the LC group, 11 of 13 participants met the criteria for orthostatic hypotension, with 7 of 11 individuals meeting the criteria for initial orthostatic hypotension (IOH) and 4 participants were classified as having classical orthostatic hypotension (COH). In the NLC group, 9 of 13 participants met the criteria for orthostatic hypotension, with 3 of 9 individuals meeting the criteria for IOH and 6 of 9 participants meeting the criteria for COH. Only one LC participant met the postural orthostatic tachycardia syndrome (POTS) criteria. Additionally, 6 of 13 participants in the LC group reported experiencing light-headedness/dizziness after standing, but none of the NLC participants self-reported OI symptoms. None of the participants met the criteria for delayed orthostatic hypotension (DOH).

Fisher's exact tests were used to compare the proportion of participants meeting criteria for OH, IOH, and COH between groups. No significant difference was observed in OH prevalence between the LC and NLC groups ($p = 0.645$, 95% CI: 0.361 – 16.5, odds ratio = 2.44). IOH was more frequently observed in the LC group (7/13) compared to NLC (3/13), though this difference did not reach statistical significance ($p = 0.226$; 95% CI: 0.718 – 21.1, odds ratio = 3.89). COH distribution also did not reach statistical significance between groups ($p = 0.688$; 95% CI: 0.387 – 9.60, odds ratio = 1.93).

Table 5.3. *Frequency of occurrence of orthostatic hypotension and intolerance based on the lie-to-stand protocol.*

	LC (<i>n</i> =13)	NLC (<i>n</i> =13)
OH criteria met (%)	11 (85%)	9 (69%)
IOH	7 (64%)	3 (33%)
COH	4 (36%)	6 (67%)
DOH	0	0
NBPR	2 (15%)	4 (31%)
OI symptoms reported	6 (46%)	0
POTS criteria met	1 (8%)	0

LC: long-COVID; NLC: non-long-COVID; OH: orthostatic hypotension; IOH: initial orthostatic hypotension; COH: classical orthostatic hypotension; DOH: delayed orthostatic hypotension; NBPR: normal blood pressure response; OI: orthostatic intolerance; POTS: postural orthostatic tachycardia syndrome.

Subgroup analysis

Due to 69% (*n* = 9) of our NLC participants meeting the criteria for orthostatic hypotension (OH) and 31% (*n* = 4) showing a normal blood pressure response, we conducted a subgroup analysis splitting the NLC group into two: NLC with OH (NLC_{OH}) and NLC with normal blood pressure response (NLC_{NBPR}). This disaggregation allowed us to determine whether observed physiological differences were specific to long-COVID or more broadly related to the presence of OH, regardless of COVID status. A one-way ANOVA was then conducted to compare across the three groups: LC, NLC_{OH}, and NLC_{NBPR}. None of the participants in the NLC group self-reported orthostatic intolerance (OI) symptoms.

Data reporting

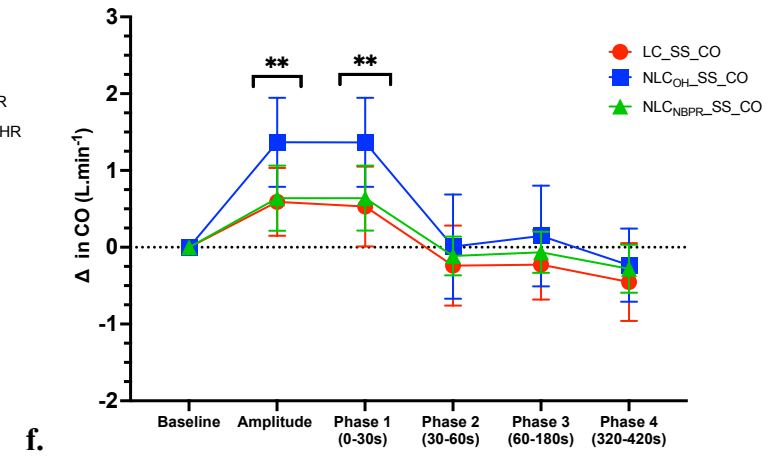
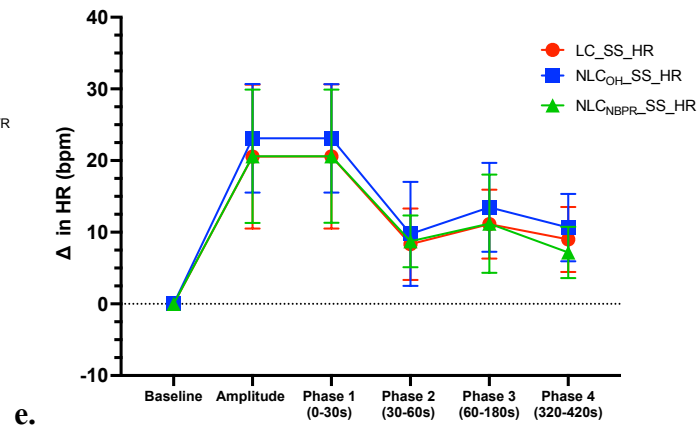
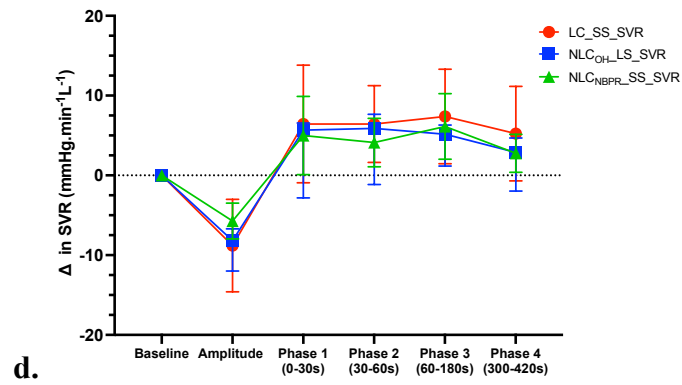
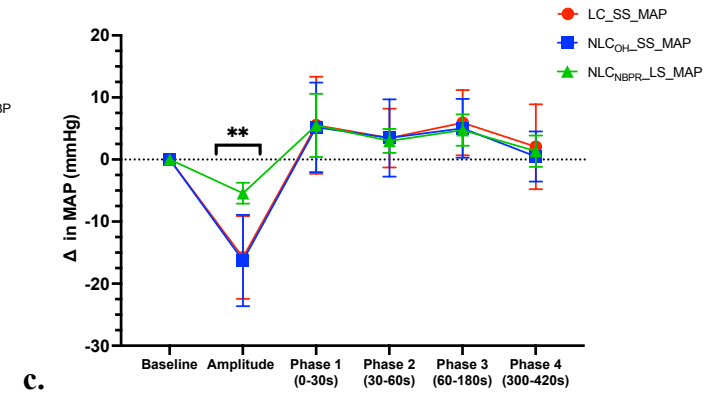
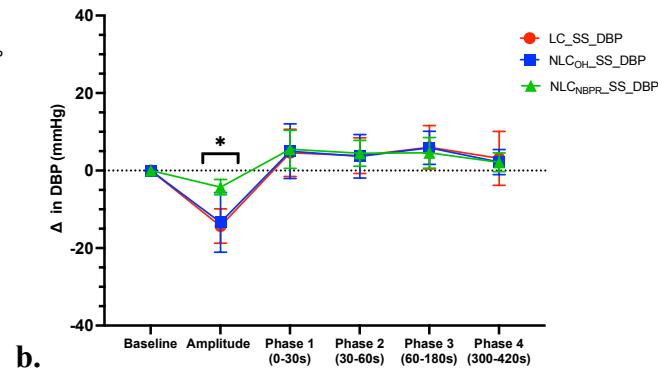
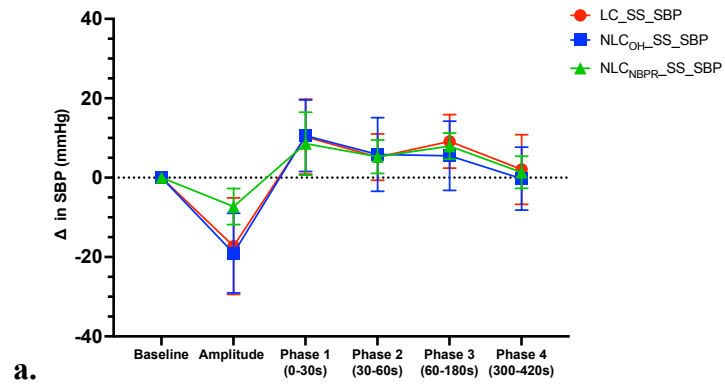
The data below are reported with both unadjusted p -values and p -values adjusted for multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (FDR). A single asterisk (*) in the graph indicates a significant difference based on the unadjusted ANOVA p -value, but not after the adjustment was applied. A double asterisk (**) indicates a significant difference in both the unadjusted and adjusted p -values.

Sit-to-stand protocol

At baseline, there were no statistically significant differences between groups in SBP, DBP, MAP, SVR, HR, CO or SV, indicating similar resting hemodynamic values. After standing, significant group differences were observed in DBP ($p = 0.015$), MAP ($p = 0.012$), and CO ($p = 0.004$) (Table 5.4). However, no significant differences were found in SBP, SVR, HR and SV.

Post-hoc comparisons showed that the significant differences were primarily between the LC *vs.* NLC_{NBPR} and NLC_{OH} *vs.* NLC_{NBPR} groups. For DBP amplitude, the LC group (14.3 ± 4.43 mmHg) and the NLC_{NBPR} (4.3 ± 1.96 mmHg) groups differed significantly ($p = 0.012$), as did the NLC_{OH} (13.4 ± 7.69 mmHg) and NLC_{NBPR} groups ($p = 0.033$), but no significant difference was found between the LC and NLC_{OH} groups ($p = 0.917$). Similarly, MAP amplitude showed significant differences between the LC group ($15.6 [6.8]$) and the NLC_{NBPR} group ($4.9 [1.3]$) ($p = 0.018$), and between the NLC_{OH} group ($15.1 [5.3]$) and the NLC_{NBPR} group ($p = 0.015$), with no significant difference between LC and NLC_{OH} ($p = 0.985$). For CO amplitude, the LC group (0.5 ± 0.52 L.min⁻¹) and NLC_{NBPR} group (0.6 ± 0.42 L.min⁻¹) showed no significant difference ($p = 0.938$). However, the LC *vs.* NLC_{OH} comparison for CO amplitude was significant ($p = 0.004$), with the LC group showing lower CO peak amplitude (0.5 ± 0.52 L.min⁻¹)

compared to the NLC_{OH} group ($1.4 \pm 0.58 \text{ L}\cdot\text{min}^{-1}$). A similar pattern was observed for CO values in phase 1 with a significant difference between LC ($0.5 \pm 0.52 \text{ L}\cdot\text{min}^{-1}$) and NLC_{OH} ($1.4 \pm 0.58 \text{ L}\cdot\text{min}^{-1}$) ($p = 0.004$), but no significant differences were found between LC and NLC_{NBPR} ($p = 0.931$) or between NLC_{OH} and NLC_{NBPR} ($p = 0.079$). Figure 5.4 shows the graphical representation of the results.



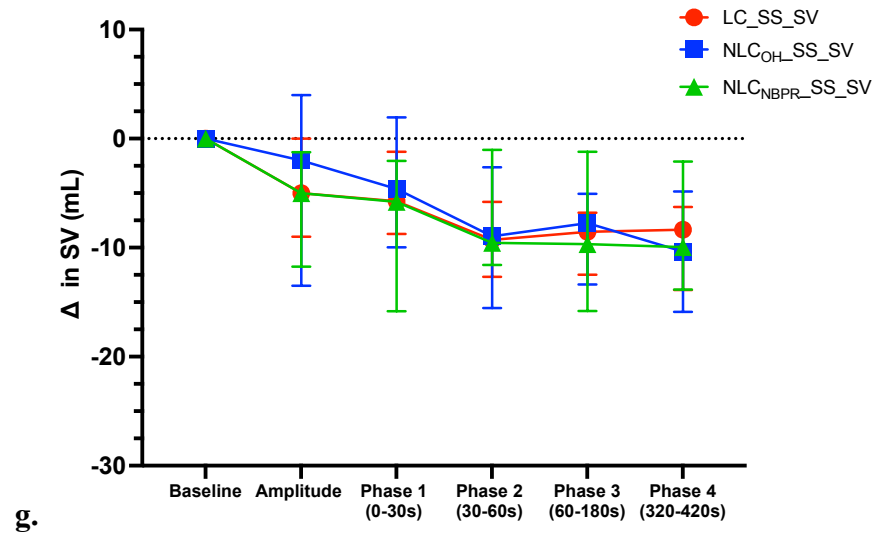


Figure 5.4: Sit-to-stand (SS) data across LC, NLC_{OH} , and NLC_{NBPR} groups presented with grouped line plot. (a) SBP (b) DBP (c) MAP (d) SVR (e) HR (f) CO (g) SV.

*Significant at p -value, but not after Benjamini-Hochberg adjustment. **significant at p -value and after Benjamini-Hochberg adjustment.

Table 5.4. Comparative evaluation of cardiovascular responses between LC, NLC_{OH}, and NLC_{NBPR} during sit-to-stand

Variable	Group	Baseline	Amplitude	Phase 1	Phase 2	Phase 3	Phase 4
SBP (mmHg)	LC	117.3 ± 9.83	-13.9 [7.0]	10.3 ± 9.44	5.2 ± 5.84	9.1 ± 6.75	2.1 ± 8.79
		(111.4; 123.3)	(-5.6; -47.3]	(4.6; 16.01)	(1.7; 8.7)	(5.1; 13.2)	(-3.3; 7.4)
	NLC _{OH}	114.3 ± 9.76	-14.1 [7.83]	10.6 ± 9.02	5.8 ± 9.28	5.5 ± 8.72	-0.2 ± 7.92
		(106.8; 121.8)	(-7.9; -36.2)	(3.7; 17.5)	(-1.3; 12.9)	(-1.2; 12.2)	(-6.3; 5.8)
	NLC _{NBPR}	117.1 ± 12.74	-8.16 [3.69]	8.6 ± 7.93	5.3 ± 4.2	7.9 ± 3.28	1.3 ± 4.05
		(96.8; 137.3)	(-1.0; -11.8)	(-4.1; 21.2)	(-1.4; 11.9)	(2.7; 13.2)	(-5.1; 7.8)
	<i>p-value</i>	0.783	0.062	0.929	0.977	0.513	0.804
	<i>Adjusted p</i>	0.977	0.217	0.977	0.977	0.977	0.977
Effect size	0.021	0.222	0.006	0.002	0.056	0.019	
DBP (mmHg)	LC	64.2 ± 10.32	-14.3 ± 4.43↓	4.6 ± 7.09	3.9 ± 4.61	6.0 ± 5.59	3.2 ± 6.95
		(57.9; 70.5)	(-11.7; -17.0)	(0.9; 8.3)	(1.1; 6.6)	(2.7; 9.4)	(-1.1; 7.4)
	NLC _{OH}	67.8 ± 7.66	-13.4 ± 7.69↓	5.0 ± 7.04	3.7 ± 5.62	5.9 ± 4.28	2.2 ± 3.24
		(61.9; 73.7)	(-7.5; -19.3)	(-0.4; 10.4)	(-0.6; 7.9)	(2.6; 9.2)	(-0.3; 4.7)

MAP (mmHg)	NLC _{NBPR}	64.8 ± 17.38 (37.1; 92.4)	-4.3 ± 1.96 (-1.2; -7.4)	5.5 ± 4.91 (-2.3; 13.3)	4.5 ± 3.3 (-0.8; 9.8)	4.6 ± 3.96 (-1.7; 10.9)	2.1 ± 2.40 (-1.7; 5.9)
	<i>p-value</i>	0.739	0.015*	0.965	0.963	0.873	0.834
	<i>Adjusted p</i>	0.965	0.053	0.965	0.965	0.965	0.965
	Effect size	0.026	0.307	0.003	0.003	0.012	0.015
	LC	82.1 ± 8.94 (76.7; 87.5)	-15.6 [6.8]↓ (-6.9; -28.9)	5.5 ± 7.82 (0.8; 10.3)	1.5 [5.8] (-1.3; 11.9)	5.9 ± 5.26 (2.8; 9.1)	2.1 ± 6.85 (-2.1; 6.2)
	NLC _{OH}	83.9 ± 7.67 (77.9; 89.8)	-15.1 [5.3]↓ (-10.5; -24.4)	5.2 ± 7.21 (-0.4; 10.7)	1.9 [5.6] (-4.6; 15.9)	5.0 ± 4.73 (1.4; 8.7)	0.5 ± 3.58 (-2.6; 3.6)
	NLC _{NBPR}	82.3 ± 18.21 (53.3; 111.3)	-4.9 [1.3] (-4.0; -7.8)	5.5 ± 5.08 (-2.6; 13.6)	2.9 [2.7] (0.9; 5.1)	4.8 ± 2.53 (0.7; 8.8)	1.4 ± 2.53 (-2.7; 5.4)
	<i>p-value</i>	0.920	0.012*	0.994	0.946	0.869	0.810
	<i>Adjusted p</i>	0.995	0.042*	0.994	0.994	0.994	0.994
	Effect size	0.007	0.357	0.001	0.004	0.012	0.018
	LC	28.4 ± 8.51	-7.9 [7.4]	3.6 [4.8]	6.4 ± 4.81	7.4 ± 5.92	5.2 ± 5.92

SVR (mmHg.min ⁻¹ L ⁻¹)		(23.3; 33.5)	(-1.9; -17.9)	(0.6; 24.7)	(3.5; 9.4)	(3.8; 10.9)	(1.7; 8.8)
	NLC _{OH}	26.5 ± 7.94	-8.2 [3.9]	5.7 [8.6]	4.2 ± 4.4	4.6 ± 4.31	2.5 ± 4.54
		(20.4; 32.6)	(-3.8; -26.5)	(-6.5; 11.6)	(0.9; 7.6)	(1.2; 7.9)	(-1.0; 5.9)
	NLC _{NBPR}	28.1 ± 8.63	-5.8 [2.4]	5.7 [6.0]	4.1 ± 3.04	6.1 ± 4.11	2.7 ± 2.38
		(14.4; 41.8)	(-3.0; -8.4)	(-1.2; 9.8)	(-0.7; 8.9)	(-0.4; 12.7)	(-1.0; 6.6)
	<i>p-value</i>	0.867	0.365	0.776	0.451	0.465	0.428
	<i>Adjusted p</i>	0.867	0.651	0.867	0.651	0.651	0.651
	Effect size	0.012	0.081	0.020	0.067	0.064	0.071
HR (bpm)	LC	67.6 ± 9.75	20.6 ± 10.06	20.6 ± 10.06	8.3 ± 4.99	11.1 ± 4.82	8.9 ± 4.53
		(61.7; 73.5)	(14.5; 26.6)	(14.5; 26.7)	(5.3; 11.3)	(8.2; 14.0)	(6.3; 11.7)
	NLC _{OH}	67.3 ± 8.31	23.1 ± 7.55	23.1 ± 7.55	9.8 ± 7.26	13.5 ± 6.21	10.6 ± 4.69
		(60.9; 73.7)	(17.3; 28.9)	(17.3; 28.9)	(4.2; 15.3)	(8.7; 18.2)	(7.0; 14.3)
	NLC _{NBPR}	65.3 ± 4.44	20.6 ± 9.29	20.6 ± 9.29	8.7 ± 3.61	11.2 ± 6.86	7.2 ± 3.58
		(58.3; 72.4)	(5.8; 35.4)	(5.8; 35.4)	(2.9; 14.5)	(0.3; 22.1)	(1.5; 12.9)
	<i>p-value</i>	0.899	0.800	0.803	0.877	0.611	0.427

	<i>Adjusted p</i>	0.899	0.899	0.899	0.899	0.899	0.899
	Effect size	0.009	0.019	0.019	0.015	0.011	0.071
CO (L.min⁻¹)	LC	3.1 ± 0.80	0.5 ± 0.52	0.5 ± 0.52	-0.04 [0.5]	-0.2 ± 0.45	-0.4 [0.3]
		(2.6; 3.6)	(0.2; -0.8)	(0.2; 0.8)	(-1.4; 0.3)	(-0.5; 0.04)	(-1.6; 0.2)
	NLC _{OH}	3.4 ± 0.96	1.3 ± 0.58 [↑]	1.4 ± 0.58 [↑]	-0.16 [0.8]	0.15 ± 0.66	-0.3 [0.6]
		(2.7; 4.2)	(0.9; -1.8)	(0.9; 1.8)	(-0.9; 1.1)	(-0.4; 0.7)	(-1.0; 0.4)
	NLC _{NBPR}	2.9 ± 0.25	0.6 ± 0.42	0.6 ± 0.42	-0.18 [0.2]	-0.07 ± 0.27	-0.4 [0.2]
		(2.6; 3.4)	(0.04; 1.3)	(-0.03; 1.3)	(-0.3; 0.2)	(-0.5; 0.4)	(-0.6; 0.2)
	<i>p-value</i>	0.772	0.004*	0.004*	0.920	0.269	0.679
	<i>Adjusted p</i>	0.901	0.009*	0.009*	0.920	0.470	0.901
	Effect size	0.033	0.377	0.377	0.007	0.108	0.031
SV (mL)	LC	40.2 [23.4]	- 4.9 [7.3]	-5.8 [5.6]	-9.3 [5.3]	-8.6 [3.4]	-8.4 [4.3]
		(30.3; 98.3)	(-61.7; 3.6)	(-60.6; 0.1)	(-47.1; -1.52)	(-55.9; -1.3)	(-53.1; -3.08)
	NLC _{OH}	47.5 [13.3]	-2.4 [14.7]	-4.6 [8.3]	-8.9 [10.3]	-7.8 [4.3]	-10.4 [9.9]
		(32.5; 79.6)	(-15.2; 7.6)	(-13.3; 5.9)	(-17.9; -0.4]	(-18.2; -1.1)	(-17.5; -3.2)

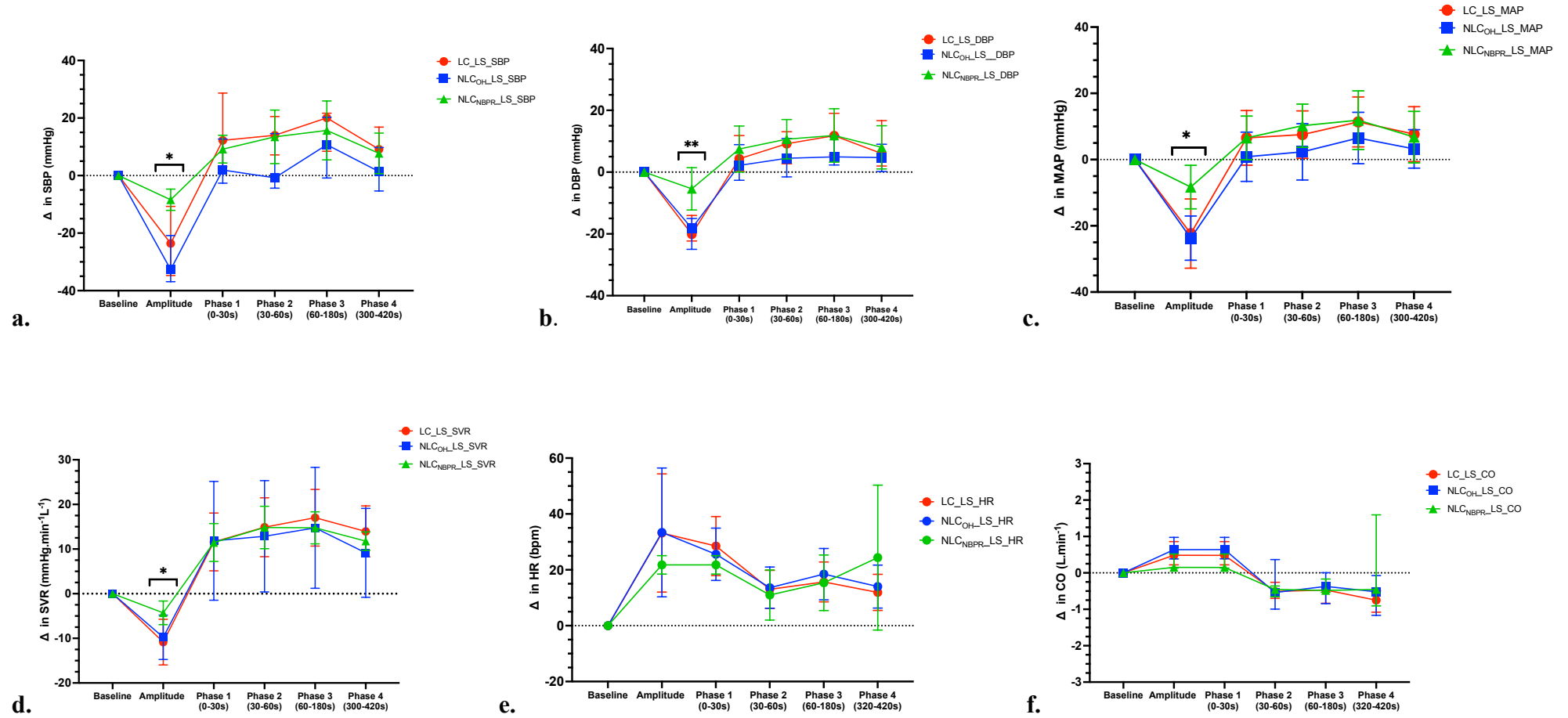
NLC _{NBPR}	47.8 [4.5]	-5.1 [7.6]	-5.8 [9.4]	-9.6 [5.6]	-9.6 [11.4]	--9.9 [6.9]
	(37.7; 50.3)	(-12.9; -1.0)	(-17.9; -2.0)	(-11.8; 1.3)	(-16.2; -0.03)	(-14.4; -0.2)
<i>p-value</i>	0.664	0.784	0.772	0.885	0.961	0.939
<i>Adjusted p</i>	0.961	0.961	0.961	0.961	0.961	0.961
Effect size	0.033	0.020	0.021	0.009	0.003	0.005

Mean $\pm$ Standard Deviation (SD) with 95% Confidence Interval of mean; Median [Interquartile range] with minimum and maximum; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; SVR: systemic vascular resistance; HR: heart rate; CO: cardiac output; SV: stroke volume; Adj p: adjusted p-value; ES: effect size. $\downarrow$ statistically lower than; $\uparrow$ statistically higher than. *Statistically significant differences between long-COVID, non-long-COVID with OH and non-long-COVID without OH groups. The variable values are expressed as Δ from baseline, where negative numbers represent below baseline values and positive numbers above baseline values.

Lie-to-stand protocol

At baseline, there were no significant differences between groups in SBP, DBP, MAP, SVR, HR, CO, or SV. However, following the transition to standing, significant group differences were observed in SBP ($p = 0.031$), DBP ($p = 0.007$), MAP ($p = 0.020$), and SVR ($p = 0.040$) (Table 5.5). *Post-hoc* comparisons showed that SBP amplitude was significantly different between the NLC_{OH} group (-29.4 ± 8.91 mmHg) and the NLC_{NBPR} (-8.4 ± 3.69 mmHg) group ($p = 0.024$), with the NLC_{OH} group showing a pronounced drop compared to the NLC_{NBPR} group, but no significant difference was found between the LC and NLC_{OH} ($p = 0.496$). DBP amplitude showed a significant difference between the LC group (-18.9 ± 7.91 mmHg) and the NLC_{NBPR} group (-5.4 ± 6.85 mmHg) ($p = 0.008$), and between the NLC_{OH} group (-19.3 ± 5.82 mmHg) and the NLC_{NBPR} group ($p = 0.010$), with the LC and NLC_{OH} groups showing a pronounced drop compared to the NLC_{NBPR} group. No significant difference was found between the LC and NLC_{OH} groups ($p = 0.990$). MAP amplitude also showed significant differences between the LC group (-22.4 ± 10.45 mmHg) and the NLC_{NBPR} group (-8.3 ± 6.57 mmHg) ($p = 0.027$), and between the NLC_{OH} group (-23.7 ± 6.68 mmHg) and the NLC_{NBPR} group ($p = 0.021$), with the LC and NLC_{OH} groups showing a more pronounced drop compared to the NLC_{NBPR} group. No significant difference was found between LC and NLC_{OH} ($p = 0.934$). For SVR, a significant difference was observed between the LC group ($-9.9 [5.3]$) and the NLC_{NBPR} group ($-5.5 [1.7]$) ($p = 0.025$), with the LC group showing a pronounced drop compared to NLC_{NBPR} . No significant difference was found between NLC_{OH} ($-10.3 [6.5]$) and NLC_{NBPR} ($p = 0.153$), and no significant difference between LC and NLC_{OH} ($p = 0.901$). No statistically significant group differences were found in HR, CO, or SV in response to

standing and across all variables phases (Table 5.5). Figure 5.5 shows graphical representation of the results.



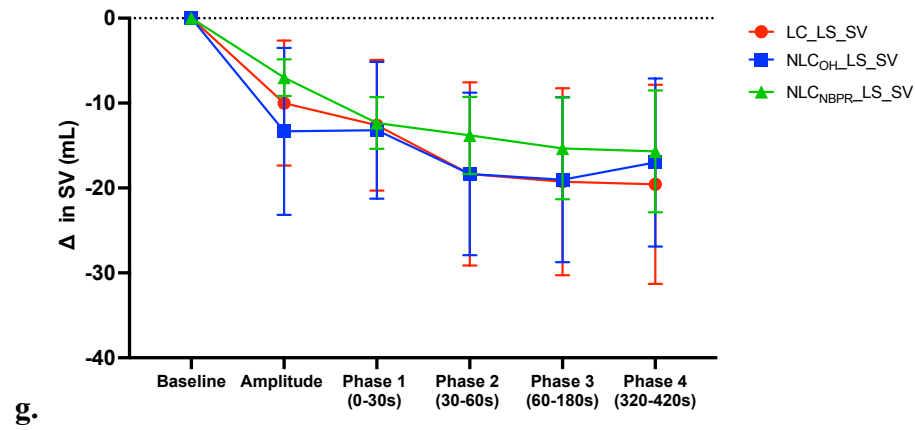


Figure 0.5: Lie-to-stand (LS) data across LC, NLC_{OH}, and NLC_{NBPR} groups presented with grouped line plot. (a) SBP (b) DBP (c) MAP (d) SVR (e) HR (f) CO (g) SV.

*Significant at p -value, but not after Benjamini-Hochberg adjustment. **Significant at p -value and after Benjamini-Hochberg adjustment.

Table 5.5. Comparative evaluation of cardiovascular responses between LC, NLC_{OH} , and NLC_{NBPR} during lie-to-stand.

Variable	Group	Baseline	Amplitude	Phase 1	Phase 2	Phase 3	Phase 4
SBP (mmHg)	LC	133.2 ± 9.35	-23.4 ± 15.4	14.8 ± 11.78	14.5 ± 8.65	16.1 ± 9.59	9.6 ± 9.14
		(127.6; 138.9)	(-14.1; -32.7)	(7.7; 21.9)	(9.2; 19.7)	(10.3; 21.9)	(4.1; 15.1)
	NLC_{OH}	121.9 ± 18.89	-29.4 ± 8.91	4.2 ± 11.45	4.2 ± 12.63	9.1 ± 12.39	4.4 ± 12.08
		(107.4; 136.5)	(-22.7; -36.4)	(-4.6; 13.0)	(-5.5; 13.9)	(-0.4; 18.7)	(-4.9; 13.7)
	NLC_{NBPR}	125.4 ± 12.19	-8.4 ± 3.69	9.2 ± 4.81	13.5 ± 9.29	15.7 ± 10.24	7.7 ± 7.04
		(105.9; 144.8)	(-2.5; -14.3)	(1.5; 16.8)	(-1.3; 28.2)	(-0.6; 31.9)	(-3.5; 18.9)
	<i>p-value</i>	0.249	0.031*	0.105	0.081	0.317	0.505
	<i>Adjusted p</i>	0.349	0.109	0.184	0.184	0.369	0.505
	Effect size	0.141	0.260	0.178	0.196	0.095	0.058
	LC	77.2 ± 9.48	-18.9 ± 7.91	6.6 ± 8.19	8.5 ± 6.88	12.0 ± 7.95	9.0 ± 8.51
		(71.4; 82.9)	(-14.1; -23.6)	(1.7; 11.5)	(4.3; 12.6)	(7.2; 16.8)	(3.9; 14.2)
	NLC_{OH}	76.6 ± 8.18	-19.3 ± 5.82	2.6 ± 6.19	4.4 ± 7.01	7.6 ± 5.79	4.6 ± 4.53
		(70.3; 82.9)	(-14.8; -23.8)	(-2.1; 7.4)	(-0.9; 9.8)	(3.1; 12.0)	(1.1; 8.1)

DBP (mmHg)	NLC _{NBPR}	73.6 ± 10.35	-5.4 ± 6.85	7.5 ± 7.46	10.7 ± 6.37	11.9 ± 8.7	8.0 ± 7.01
		(57.1; 90.0)	(-5.5; -16.3)	(-4.4; 19.3)	(0.5; 20.8)	(-1.9; 25.6)	(-3.1; 19.2)
	<i>p-value</i>	0.791	0.007*	0.407	0.250	0.363	0.366
	<i>Adjusted p</i>	0.791	0.025*	0.475	0.475	0.475	0.475
	Effect size	0.020	0.351	0.075	0.114	0.084	0.084
MAP (mmHg)	LC	94.6 [11.4]	-22.4 ± 10.45	6.6 ± 8.25	7.6 ± 7.16	11.4 ± 7.54	7.7 ± 8.28
		(77.4; 106.9)	(-16.0; -28.7)	(1.5; 11.5)	(3.2; 11.5)	(6.8; 15.9)	(2.7; 12.7)
	NLC _{OH}	92.1 [15.1]	-23.7 ± 6.68	0.8 ± 7.44	2.3 ± 8.51	6.5 ± 7.76	3.2 ± 5.81
		(78.8; 111.8)	(-18.6; -28.8]	(-4.9; 6.6)	(-4.2; 8.9)	(0.6; 12.5)	(-1.2; 7.7)
	NLC _{NBPR}	86.9 [6.3]	-8.3 ± 6.57	6.6 ± 6.59	10.2 ± 6.52	11.9 ± 8.85	6.8 ± 7.77
		(84.5; 109.4)	(-2.2; -18.8]	(-3.9; 17.1)	(-0.1; 20.6)	(-2.2; 25.9)	(-5.6; 19.1)
	<i>p-value</i>	0.535	0.020*	0.225	0.166	0.318	0.390
	<i>Adjusted p</i>	0.535	0.070	0.394	0.445	0.445	0.455
	Effect size	0.050	0.290	0.122	0.145	0.095	0.079
	LC	33.3 [13.8]	-9.9 [5.3]	11.6 ± 6.46	14.9 ± 6.59	17.0 ± 6.34	13.9 ± 5.71

SVR (mmHg.min ⁻¹ L ⁻¹)		(19.3; 57.8)	(-2.1; -20.7)	(7.7; 15.5)	(10.9; 18.9)	(13.2; 20.9)	(10.5; 17.4)
	NLC _{OH}	26.7 [10.3]	-10.3 [6.5]	11.8 ± 13.31	12.9 ± 12.47	14.7 ± 13.54	9.2 ± 9.96
		(21.5; 40.2)	(-2.1; -18.1)	(1.6; 22.1)	(3.3; 22.5)	(4.3; 25.2)	(1.5; 16.8)
	NLC _{NBPR}	29.7 [2.9]	-5.5 [1.7]	11.5 ± 4.25	14.8 ± 4.75	14.8 ± 3.58	11.8 ± 2.06
		(28.4; 39.1)	(-0.3; -5.9)	(-5.6; 19.2)	(7.3; 22.4)	(9.1; 20.5)	(8.5; 15.1)
	<i>p-value</i>	0.608	0.040*	0.997	0.861	0.825	0.369
	<i>Adjusted p</i>	0.997	0.140	0.997	0.997	0.997	0.861
	Effect size	0.039	0.257	0.000	0.013	0.017	0.093
HR (bpm)	LC	63.4 [6.3]	28.5 ± 10.56	28.5 ± 10.56	13.0 ± 6.81	17.9 [10.8]	11.9 ± 6.47
		(38.5; 71.5)	(22.1; 24.9)	(22.1; 34.9)	(8.9; 17.1)	(3.1; 26.3)	(8.0; 15.8)
	NLC _{OH}	61.3 [9.3]	25.6 ± 9.38	25.6 ± 9.37	13.6 ± 7.37	18.9 [9.4]	13.9 ± 7.69
		(55.7; 78.7)	(15.7; 51.1)	(18.4; 32.8)	(7.9; 19.3)	(3.1; 32.9)	(8.1; 19.9)
	NLC _{NBPR}	63.1 [13.0]	21.8 ± 3.30	21.8 ± 3.30	10.9 ± 8.96	10.6 [5.4]	24.4 ± 25.9
		(52.8; 71.2)	(16.5; 27.0)	(16.5; 27.0)	(-3.3; 25.2)	(9.9; 30.3)	(-16.9; 65.6)
	<i>p-value</i>	0.979	0.448	0.448	0.834	0.630	0.590

	<i>Adjusted p</i>	0.979	0.979	0.979	0.979	0.979	0.979
	Effect size	0.001	0.067	0.067	0.016	0.037	0.138
CO (L.min⁻¹)	LC	3.1 ± 0.71	0.5 [0.4]	0.5 [0.4]	-0.5 ± 0.44	-0.6 ± 0.37	-0.8 ± 0.47
		(2.6; 3.5)	(0.06; 3.1)	(0.06; 3.1)	(-0.8; -0.3)	(-0.8; 0.3)	(-1.1; -0.5)
	NLC _{OH}	3.3 ± 0.72	0.6 [0.4]	0.6 [0.4]	-0.5 ± 0.82	0.4 ± 0.77	-0.6 ± 0.78
		(2.7; 3.8)	(-0.3; 1.9)	(-0.3; 1.9)	(-1.1; 0.2)	(-1.0; 0.2)	(-1.2; 0.03)
	NLC _{NBPR}	2.9 ± 0.11	0.2 [0.1]	0.2 [0.1]	-0.5 ± 0.16	-0.39 ± 0.22	0.1 ± 1.44
		(2.7; 3.1)	(0.1; 0.6)	(0.1; 0.6)	(-0.7; -0.2)	(-0.7; -0.05)	(-2.2; 2.4)
	<i>p-value</i>	0.645	0.245	0.249	0.948	0.785	0.464
	<i>Adjusted p</i>	0.903	0.858	0.858	0.948	0.916	0.858
	Effect size	0.037	0.112	0.111	0.002	0.021	0.145
SV (mL)	LC	50.9 ± 16.79	-10.1 ± 7.33	-12.6 ± 7.69	-16.2 [8.5]	-15.7 [8.0]	-19.6 ± 11.7
		(40.8; 61.1)	(-14.6; -5.7)	(-17.3; -7.9)	(-39.3; -3.7)	(-42.6; -2.9)	(-26.7; -12.5)
	NLC _{OH}	52.0 ± 13.1	-13.3 ± 10.01	-13.2 ± 8.06	-16.1 [5.1]	-17.4 [6.9]	-16.9 ± 9.89
		(41.9; 62.1)	(-20.9; -5.6)	(-19.4; -7.00)	(-42.1; -17.6]	(-42.1; -7.5)	(-24.6; -9.4)

NLC _{NBPR}	47.5 ± 8.6	-7.1 ± 2.11	-12.3 ± 3.04	-11.3 [2.6]	-17.3 [4.7]	-15.7 ± 7.18
	(33.9; 61.2)	(-10.5; -3.8)	(-17.2; -7.5)	(-16.8; -10.1)	(-20.1; -6.6)	(-27.1; -4.3)
<i>p-value</i>	0.877	0.170	0.975	0.770	0.935	0.760
<i>Adjusted p</i>	0.975	0.595	0.975	0.975	0.975	0.975
Effect size	0.011	0.072	0.002	0.021	0.005	0.024

Mean ± Standard Deviation (SD) with 95% Confidence Interval of mean; Median [Interquartile range] with minimum and maximum; SBP:

systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; SVR: systemic vascular resistance; HR: heart rate; CO:

cardiac output; SV: stroke volume; Adj p: adjusted p-value; ES: effect size. ↓ significantly lower than; ↑ significantly higher than. *Statistically

significant differences between long-COVID, non-long-COVID with OH and non-long-COVID without OH groups. The variable values are

expressed as Δ from baseline, where negative numbers represent below baseline values and positive numbers above baseline values.

Cardiac Output and Systemic Vascular Resistance Matching

Sit-to-stand

Spearman's rank correlation was conducted for phases 1-4 to assess the relationship between cardiac output (CO) and systemic vascular resistance (SVR). Across all groups, phase 1 showed a very low non-significant negative correlation (Spearman's $r = -0.249$, $p = 0.219$), while phase 2 showed a moderate significant negative correlation ($r = -0.681$; $p < 0.001$). Phases 3 and 4 showed high significant negative correlations (phase 3: $r = -0.804$; $p < 0.001$; phase 4: $r = -0.819$, $p < 0.001$).

In the LC group, phase 1 showed a very low negative correlation that was not statistically significant ($r = -0.236$; $p = 0.437$). Phase 2 showed a moderate significant negative correlation ($r = -0.670$, $p = 0.015$), while phases 3 and 4 showed high ($r = -0.791$; $p = 0.002$) and very high ($r = -0.890$; $p < 0.001$) significant negative correlations, respectively. The NLC_{OH} group showed high negative correlations, which was statistically significant across all phases (phase 1 $r = -0.767$, $p = 0.021$; phase 2 $r = -0.800$, $p = 0.014$; phase 3 $r = -0.850$; $p = 0.006$; phase 4 $r = -0.800$, $p = 0.014$). In the NLC_{NBPR} group, phase 1 showed a moderate positive correlation ($r = 0.600$; $p = 0.417$), while phases 2, 3 and 4 showed high negative correlations (phase 2 $r = -0.800$, $p = 0.333$; phase 3 $r = -0.800$; $p = 0.333$; phase 4 $r = -0.800$; $p = 0.333$), none of which were statistically significant. Figure 5.6 shows CO-SVR correlation across phases for the LC, NLC_{OH}, and NLC_{NBPR} groups.

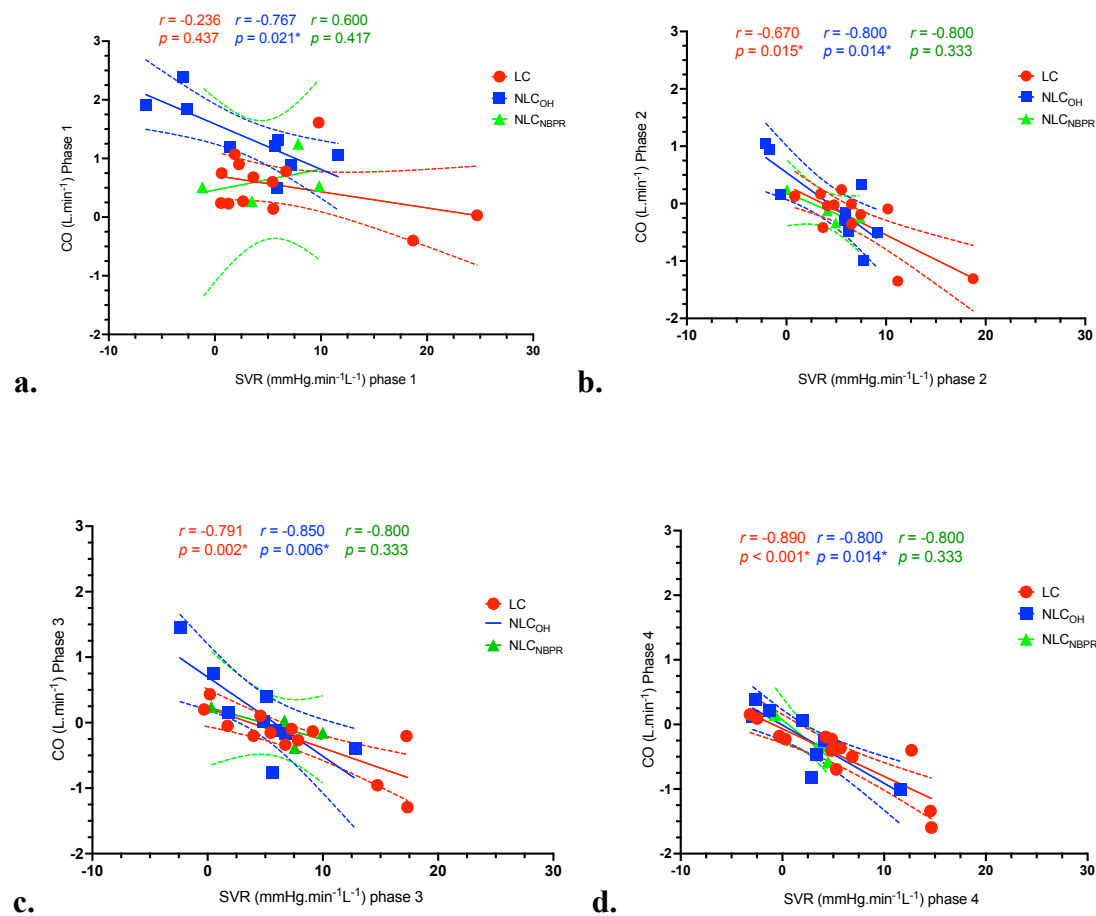


Figure 5.6: Cardiac output–systemic vascular resistance matching data across phases

1-4 during sit-to-stand (SS) across LC, NLC_{OH}, and NLC_{NBPR} groups.

Lie-to-stand

Pearson's rank correlation was conducted for phases 1, 2, 3, and 4 to assess the relationship between cardiac output (CO) and systemic vascular resistance (SVR). Across all groups, phase 1 showed a very low negative correlation that was not statistically significant (Pearson's $r = -0.071$, 95% CI: -0.446 to 0.325, $p = 0.730$). In contrast, phases 2 and 3 showed high negative correlations that were statistically significant (phase 2: $r = -0.751$, 95% CI: -0.882 to -0.512, $p < 0.001$; phase 3: $r = -0.842$, 95% CI: 0.927 to -0.674, $p < 0.001$), and phase 4 showed a moderate and statistically significant negative correlation ($r = -0.580$; 95% CI: -0.790 to -0.248, $p = 0.002$).

For the LC group, phase 1 showed a low positive correlation that was not statistically significant ($r = 0.332$, 95% CI: -0.268 to 0.746, $p = 0.268$). Phases 2, 3 and 4 showed moderate negative correlations that were not statistically significant (phase 2: $r = -0.466$, 95% CI: -0.809 to 0.115, $p = 0.109$; phase 3: $r = -0.598$; 95% CI: -0.864 to -0.071, $p = 0.031$; phase 4: $r = -0.502$; 95% CI: -0.825 to 0.068, $p = 0.080$). The NLC_{OH} group showed a moderate negative correlation in phase 1 that was not statistically significant ($r = -0.553$, 95% CI: -0.890 to 0.175, $p = 0.122$), and high significant negative correlations in phases 2, 3 and 4 (phase 2: $r = -0.887$, 95% CI: -0.976, to -0.542, $p = 0.001$; phase 3: $r = -0.927$; 95% CI: -0.985 to -0.684, $p < 0.001$; phase 4: $r = -0.952$, 95% CI: -0.990 to -0.784, $p < 0.001$). For the NLC_{NBPR} group, all phases showed a moderate negative correlation that was not statistically significant (phase 1: $r = -0.690$, 95% CI: -0.993 to 0.805, $p = 0.310$; phase 2: $r = -0.472$, 95% CI: -0.986 to 0.895, $p = 0.528$; phase 3: $r = -0.607$; 95% CI: -0.990 to 0.850, $p = 0.393$; phase 4: $r = -0.320$, 95% CI: to -0.980 to 0.926, $p = 0.680$). Figure 5.7 shows CO-SVR correlation across phases for the LC, NLC_{OH}, and NLC_{NBPR} groups.

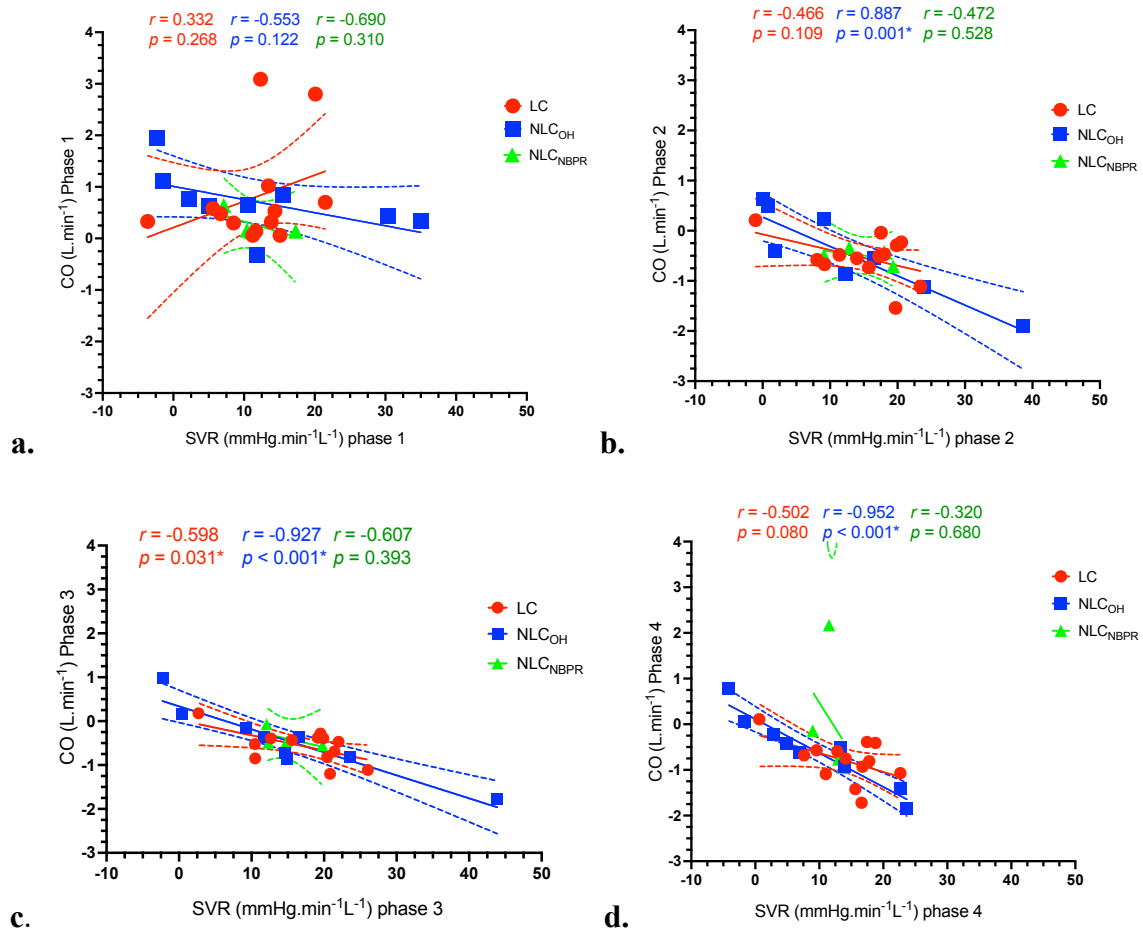


Figure 5.7: Cardiac output–systemic vascular resistance matching data across phases 1-4 during lie-to-stand (LS) across LC, NLC_{OH}, and NLC_{NBPR} groups.

Discussion

The purpose of this study was to characterize and compare the frequency of occurrence of orthostatic hypotension (OH) and orthostatic intolerance (OI) symptoms between individuals with and without long-COVID, assess symptom severity and frequency using the DePaul Symptom Questionnaire- short form (DSQ-SF), and examine short-term cardiovascular compensatory responses (SBP, DBP, MAP, SVR, HR, CO and SV) across four time phases during an active standing orthostatic stress test. Our findings support the hypothesis that long-COVID is associated with increased symptom burden, autonomic dysfunction and altered cardiovascular regulation.

Frequency of occurrence of OH and OI symptoms

A high incidence of dysautonomia, including OH, postural orthostatic tachycardia syndrome, and OI symptoms, has been widely reported in individuals with long-COVID (Dani et al., 2021; Lee et al., 2024; Monaghan et al., 2022). In our sample, 85% ($n = 11$ of 13) of participants in our long-COVID group met the criteria for OH, and 46% reported OI symptoms after standing. Interestingly, while none of the participants in the non-long-COVID (NLC) group reported OI symptoms after standing, 69% ($n = 9$ of 13) still met the criteria for OH. This dissociation between objective hemodynamic changes and subjective symptom experience might be dependent on a more effective compensatory mechanism in the NLC group, such as the ability to maintain cerebral perfusion or efficient match in CO-SVR despite a drop in blood pressure (Campen & Visser, 2022; Khan et al., 2025; Wehrwein & Joyner, 2013). In contrast, individuals with long-COVID may experience impairments in these compensatory mechanisms, resulting in greater symptom burden (Buoite Stella et al., 2022; Khan et al., 2025; Wold et al., 2024). This finding suggests the possibility that OH is not unique to long-COVID and also raises the

possibility that some individuals in the NLC group may have experienced asymptomatic or undiagnosed SARS-CoV-2 infections. This is plausible given that early in the pandemic, limited testing capacity and high rates of asymptomatic spread may have led to unrecognized infections (Parasher, 2021; Raveendran, 2021).

Additionally, when examining the OH responses, we found that the type of OH varied across groups. In the long-COVID group, 64% ($n = 7$ of 11) met the criteria for initial orthostatic hypotension (IOH), characterized by a rapid drop in SBP of at least 40 mmHg or DBP of at least 20 mmHg within 30 seconds of standing, compared to only 33% ($n = 3$ of 9) in the NLC group. In contrast, classical OH (COH), defined as a drop in SBP of at least 20 mmHg or DBP of at least 10 mmHg within 3 minutes of standing, was more prevalent in the NLC group (67%, $n = 6$ of 9) compared to the LC group (36%, $n = 4$ of 11). The higher frequency of occurrence of IOH in the LC group aligns with emerging literature suggesting that IOH may be more prevalent in individuals with long-COVID (Hira et al., 2023, 2025; Monaghan et al., 2022). Underlying pathophysiology of IOH has been suggested not to be associated with autonomic failure but rather reflect a transient mismatch between cardiac output and systemic vascular resistance immediately after standing, potentially due to impaired vascular tone or delayed baroreflex activation (Fedorowski et al., 2022; Stewart & Clarke, 2011; Wieling et al., 2007). However, there remains limited reporting of IOH in this population, likely due to methodological limitations, as often studies rely on manual blood pressure measurements, which are not suitable for detecting rapid BP fluctuations, a primary characteristic of IOH (Finucane et al., 2019; Stewart & Clarke, 2011; Wieling et al., 2007).

Interestingly and for contextualization, individuals with related conditions to long-COVID such as myalgia encephalomyelitis/chronic fatigue syndrome (ME/CFS), a condition

which shares symptomatic and physiological overlap with long-COVID (Komaroff & Lipkin, 2023), have also been reported to show an high incidence of OH responses (Lee et al., 2020; Vernon et al., 2022), suggesting that OH may be part of a broader autonomic profile common to post-viral syndromes. Regarding the individuals in the NLC group who met the OH criteria but did not report symptoms, their patterns could reflect cases of asymptomatic orthostatic hypotension (OH), where individuals experience diagnostic drops in blood pressure without reporting orthostatic intolerance symptoms (Lee et al., 2024; Ricci et al., 2015). This may be due to residual autonomic changes from mild or asymptomatic infections (Bliddal et al., 2021; Jammoul et al., 2023; Lee et al., 2024), although only 23% ($n = 3$) of the NLC participants reported having tested positive for SARs-CoV-2 (with no persistent symptoms). Emerging evidence suggests that even those with mild or asymptomatic infection or overt long-term symptoms may present with autonomic dysfunction beyond the acute phase (DePace & Colombo, 2022; Parhizgar et al., 2023). However, the absence of symptom reporting, combined with a more gradual blood pressure drop, may suggest more effective compensatory mechanisms, such as preserved cerebral autoregulation or a greater physiological tolerance to transient hypotension (Khan et al., 2025; Stewart, 2012). Asymptomatic OH has been reported in older adults and clinical populations, however, its prevalence in post-viral syndromes remains unknown (Arbogast et al., 2009; Claffey et al., 2022; Freeman et al., 2020; Merola et al., 2016).

DSQ-SF symptom severity and frequency

While OI symptoms were reported by individuals in the LC group and not participants in the NLC group, further analysis using the DePaul Symptom Questionnaire – short form (DSQ-SF) characterized the broader symptom burden associated with long-COVID. The LC group rated significantly higher on the DSQ-SF symptom severity and frequency scale in 11 of the 14-

item compared to the NLC group, including fatigue, next day soreness or fatigue, tiredness after minimum exercise, feeling unrefreshed after sleep, muscle pain or aches, problem remembering things, difficulty paying attention, unsteadiness while standing, cold limbs, and feeling of sickness from smells. These symptoms, although only one participant met the Canadian Consensus Criteria for ME/CFS, closely resemble the ME/CFS symptom profile and are thought to reflect ongoing autonomic dysregulation, impaired cerebral perfusion, and neuroinflammation, all of which may reduce the ability to function at full daily capacity (Jason & Dorri, 2023; Komaroff & Lipkin, 2023; Vernon et al., 2023). However, the DSQ-SF results in our study, therefore, contributes to the growing body of evidence suggesting that long-COVID may represent a post-viral condition characterized by persistent physical and cognitive impairments that significantly impact daily functioning.

Short-term cardiovascular compensatory responses across phases

In addition to the assessment of symptom profiles, beat-by-beat cardiovascular responses were measured in individuals with long-COVID from baseline to nadir and across four distinct phases of an active stand protocol. Although previous studies have investigated cardiovascular responses to orthostatic stress in long-COVID populations (Hira et al., 2023, 2025; Monaghan et al., 2022; Seeley et al., 2023), this is the first study, to the best of our knowledge, to assess a comprehensive set of hemodynamic variables including SBP, DBP, MAP, SVR, HR, CO, SV, and CO-SVR matching across distinct time phases during both sit-to-stand and lie-to-stand orthostatic stress protocols, with the goal of providing a more detailed understanding of the timing and trajectory of cardiovascular responses to orthostatic stress in individuals with long-COVID.

At baseline, no significant differences in SBP, DBP, MAP, SVR, HR, CO, and SV were observed between the LC group and the NLC_{OH} and NLC_{NBPR} groups. However, immediately upon standing, the LC and NLC_{OH} group showed a statistically significant drop in DBP, MAP, and CO during the sit-to-stand protocol, and a drop in SBP, DBP, MAP and SVR during the lie-to-stand. While previous studies have suggested that individuals with long-COVID may demonstrate an increased sympathetic activity, which is often associated with an increased resting heart rate due to adrenergic influences (Dani et al., 2021; DePace & Colombo, 2022; Stute et al., 2021), this was not observed in our sample. Despite the more pronounced blood pressure drop observed, heart rate (HR), a primary compensatory variable (Laranjo et al., 2017), was not found to differ significantly across groups in either protocol. This pattern is consistent with previous research suggesting a blunted heart rate response in the LC group, and may reflect a potential impairment in baroreflex sensitivity, as the drop in blood pressure is not matched by an appropriate sympathetically mediated increase in heart rate (Laranjo et al., 2017; Stewart, 2012; Yılmaz & Mirzaoglu, 2025). These findings are also consistent with recent studies that show significant differences in SBP after standing in individuals with long-COVID, without a corresponding change in HR (Hira et al., 2025; Seeley et al., 2023). This blunted heart rate, despite the hypotensive response, supports the hypothesis of an underlying autonomic dysfunction involving a compromised ability of the heart to adapt to physiological demands (Serviente et al., 2022; Yılmaz & Mirzaoglu, 2025).

The drop in DBP observed following standing was also significantly pronounced in both LC and NLC_{OH} groups compared to the NLC_{NBPR} group, and this pattern was consistent across both sit-to-stand and lie-to-stand protocols. This finding is consistent with some recent studies reporting similar reductions in DBP in individuals with long-COVID (Hira et al., 2025;

Monaghan et al., 2022), although other researchers have reported contrasting results with a transient increase in DBP after standing in long-COVID participants (González-Hermosillo et al., 2023; Kato et al., 2024). It is, however, important to note that these discrepancies may be as a result of methodological differences. For example, Monaghan et al.'s (2022) within-group comparison of individuals with and without orthostatic intolerance symptoms upon standing rather than comparison to a healthy control group, as well as González-Hermosillo et al.'s (2023) use of head-up tilt testing rather than active standing (González-Hermosillo et al., 2023; Monaghan et al., 2022). However, in a healthy individual, DBP is expected to remain relatively stable or slightly increase during the transition to standing due to compensatory vasoconstriction driven by baroreflex mediated sympathetic activation (Laranjo et al., 2017; Wieling et al., 2022; Wieling & Groothuis, 2012). This compensatory response aids in maintaining vascular resistance and stabilize mean arterial pressure, and therefore, the significant drop in DBP observed in both the LC and NLC_{OH} groups might be due to a less effective and efficient compensatory mechanism (Smith et al., 1994; Tanaka et al., 1996; Wehrwein & Joyner, 2013). However, although there is limited data on how DBP responds during active standing in people with long-COVID, the underlying causes are likely similar to those that affect SBP. Upon standing, if there is not enough vasoconstriction to compensate for the drop in central blood volume, it is plausible that both SBP and DBP falls as a result of an impaired vascular compensation (Abed et al., 2012; McJunkin et al., 2015; Moya et al., 2009).

During both the sit-to-stand and lie-to-stand protocols, the LC and NLC_{OH} groups showed a significantly greater drop in MAP compared to the NLC_{NBPR} group, with no significant difference between the LC and NLC_{OH} groups. This suggests that both groups experienced more substantial hypotensive responses to orthostatic stress, suggesting venous pooling in the lower

extremities and a transient failure to adequately maintain blood pressure through compensatory mechanisms (Smith et al., 1994; Wieling & Groothuis, 2012). A similar drop in MAP during standing was reported in Hira et al. (2025), although the significant difference in their analysis was only observed between individuals with long-COVID without cardiac abnormalities and those with initial orthostatic hypotension (Hira et al., 2025). However, it is important to note that Hira's study did not provide nadir values, instead, a single average for the entire standing protocol was used, limiting direct comparisons with this current study protocol.

The lack of adequate vasoconstriction is more evident in our SVR findings during the lie-to-stand protocol, where significantly lower SVR values were observed in the LC group compared to the NLC_{NBPR} group. This suggests a reduced vasoconstrictive response and impaired vascular compensation, potentially due to increased arterial stiffness or autonomic dysregulation, both of which has been observed and reported following the acute phase of SARS-CoV-2 infection (Dani et al., 2021; Podrug et al., 2023; Saz-Lara et al., 2023; Smith et al., 1994; Szeghy et al., 2022). In contrast, SVR differences were not observed during the sit-to-stand protocol, which may indicate that vascular tone may have been less challenged during this lower-gravitational transition or that the magnitude of compensatory demand was smaller (Juraschek et al., 2022; Shaw et al., 2017).

Interestingly, however, cardiac output (CO) peak was significantly different across groups during the sit-to-stand protocol, with the NLC_{OH} group showing a significantly higher peak compared to the LC group. No significant difference in CO was found across groups during the lie-to-stand protocol. At first glance, it might seem surprising that group differences in CO were more apparent during sit-to-stand, which is considered a lower gravitational orthostatic challenge, but not during lie-to-stand (Juraschek et al., 2022). However, timing during transitions

likely played a role in this result (O'Connor et al., 2020). In this study, the average transition time for sit-to-stand was between 1-2 seconds, while the lie-to-stand transition took approximately 4-5 seconds. This faster and more abrupt posture change during sit-to-stand likely caused a more rapid drop in venous return and preload (O'Connor et al., 2020). In contrast, the relatively slower transition during lie-to-stand may have allowed more time for anticipatory sympathetic adjustments that maintained cardiac output, potentially explaining the lack of group differences during the lie-to-stand (O'Connor et al., 2020).

The higher peak in CO in the NLC_{OH} group suggests a stronger compensatory response to a rapid drop in venous return, likely due to stronger baroreflex sensitivity (Borst et al., 1984; Smith et al., 1994; Vernon et al., 2022). Also interestingly, CO peak values between the LC and NLC_{NBPR} groups were comparable, but likely for different reasons. In the NLC_{NBPR} group, it is likely that the absence of a pronounced drop in BP reduced the need for an exaggerated compensatory increase in CO. In contrast, despite a pronounced drop in BP in the LC group, CO did not increase, potentially influenced by the blunted HR response.

Stroke volume was not significantly different across groups during either protocol in our study. While there are currently no published data reporting this outcome in long-COVID populations for comparison, similar studies reporting on ME/CFS population suggest an impaired regulation of stroke volume (Nunes et al., 2023; Van Campen et al., 2024) and have been associated with reduced cerebral blood flow and the presence of orthostatic intolerance symptoms after standing (Nunes et al., 2023; Tanaka et al., 1996; Van Campen et al., 2018).

Short-term cardiovascular compensatory phases across all other measured variables did not differ significantly between groups. To date, no studies have directly compared the time course adjustments of multiple cardiovascular variables in individuals with long-COVID

compared to healthy controls using an active standing orthostatic stress protocol. Also, existing studies on long-COVID often rely on manual or automated blood pressure devices, which lack beat-by-beat data needed for detailed phase-specific analysis and time resolution, limiting direct comparisons with the findings of this study. The only phase-specific difference observed was in cardiac output during phase 1 of the sit-to-stand protocol in the NLC_{OH} group, but as mentioned above, this suggests that rapid drop in venous return caused by the abrupt and faster postural transition speed during sit-to-stand, as phase 1 corresponds with the initial time window of amplitude drop. The lack of significant group differences across phases may suggest similar short-term recovery patterns following orthostatic challenge. However, this result should be interpreted with caution, given the small sample size and warrants replication with a larger sample to improve generalizability.

CO-SVR matching responses across phases

To explore how blood pressure was regulated over time, the relationship between CO and SVR was examined across the four postural transition phases. Since MAP is influenced by both CO and SVR (Borst et al., 1984; Stewart & Clarke, 2011; Van Wijnen et al., 2016), the correlation between these two variables could provide an understanding of the timing and coordination of CO and SVR for BP regulation during active standing. However, due to the novelty of this analysis in the context of long-COVID, the interpretation of the results focuses solely on the observed patterns.

During the sit-to-stand protocol, the LC group showed a negative correlation between CO and SVR from phases 1-4, with phase 1 being statistically non-significant, and phases 2-4 being statistically significant. This suggests a delayed but eventually coordinated, compensatory response to maintain BP, achieving matching between CO-SVR after 30s (in phase 2). In

contrast, the NLC_{OH} group showed a statistically significant negative correlation across all phases, indicating an earlier and more synchronized autonomic adjustment between cardiac and vascular systems. The NLC_{NBPR} group showed a non-significant positive correlation in phase 1 and negative correlation in phases 2-4 that was not statistically significant. However, given the particularly small sample size in the NLC_{NBPR} group ($n = 4$), there's a limitation to interpreting this pattern due to a reduced reliability (Aggarwal & Ranganathan, 2016). During the lie-to-stand protocol, the LC group showed a statistically nonsignificant negative correlation across all phases, suggesting delayed coordination, while the NLC_{OH} group showed a more consistent pattern with non-significant negative correlation in phase 1 and significant negative correlations in phases 2-4. The NLC_{NBPR} group also showed non-significant negative correlation in phases 1 to 4, but caution is required in the interpretation of these NLC_{NBPR} correlation pattern due to the small sample size.

Overall, these findings suggest that while all groups eventually demonstrated some degree of CO-SVR matching, the LC group demonstrated delayed cardiovascular coordination, particularly during phase 1 (0-30s), potentially due to underlying cardio-autonomic impairment (Jammoul et al., 2023; Wehrwein & Joyner, 2013; Wieling & Karemaker, 2013). In contrast, the NLC_{OH} group, despite meeting OH criteria, showed a more rapid and coordinated adjustment, potentially due to an intact or heightened baroreflex sensitivity responding quickly to the drop in BP (Borst et al., 1984; Sprangers et al., 1991; Tanaka et al., 1996). This delayed matching in the LC group may also contribute to the observed higher incidence of OH (85%) and OI symptoms (46%) reported in this study. Disruptions in CO-SVR matching, particularly during early phase of standing, may play a central role in impaired BP stabilization and the manifestation of OI symptoms in long-COVID.

Drawing on participants' experiences living with long-COVID

Although this study was not designed to collect qualitative data, participants in the long-COVID group shared comments about their experiences living with long-COVID during the casual conversations that followed the consent process. Given that long-COVID remains an emerging and evolving condition, and that the term was itself coined by long-COVID community (Callard & Perego, 2021; Ely et al., 2024), many organizations such as the long-COVID web, have adopted a patient-centered approach when addressing this condition. In the same vein, we believe in the value of recognizing the voices of those affected, even within the frame of a quantitative study. These informal discussions were not planned or prompted by interview questions, neither were they systematically recorded or analyzed in formal qualitative methods. However, these experiences offer glimpses into the lived realities of this condition. These spontaneous conversations are presented not as formal data, but rather to share general themes that emerged and serve to humanize the findings in this study. No identifiable information and direct quotes are reported as this was not covered in our ethics approval. Several recurring themes stood out across participants including an inability to return to pre-COVID activity levels, fluctuating health marked in terms of “good days” and “bad days”, feelings of isolation and the role of online communities (social media groups), as well as frustration with the condition being misunderstood by family and medical professionals.

11 of 13 participants in the LC group described being unable to resume the physical and cognitive activities they engaged in prior to the infection. For instance, three participants had to stop working entirely. One participant in their mid-20s, a high school physical education teacher, shared that they could no longer keep up with the physical demands of their role. Others reported reducing their work hours or shifting to less demanding roles due to overwhelming

cognitive/brain fog. 10 participants also described a significant struggle returning to physical activity routines, reporting that there seemed to be a post-exertional symptom exacerbation and an overwhelming feeling of fatigue, even after minimal exercise. In connection to this, participants also emphasized the unpredictable nature of their health. They described feeling relatively well one day, trying to resume normal activities as they would have done prior to COVID, and then be completely debilitated the next day. This particularly made it difficult to plan activities ahead and contributed to feelings of distress and uncertainty about full recovery. Some also reported learning to pace themselves even on days when they felt better than usual.

Another common experience participants reported was a sense of isolation, especially early on of the condition when they felt misunderstood and dismissed by others, even doctors, especially given the invisibility of many of their symptoms. One participant specifically expressed grief over not being believed by their own family at the onset of their condition. Participants, however, all mentioned that over time, they found support through online communities, particularly Facebook groups, where they connected with others going through similar struggles. These online communities became essential, especially for building a sense of community through shared experience.

Importance of the study and recommendations for future research

The findings of this study contributes to the body of research on the cardiovascular consequences of long-COVID by assessing beat-by-beat hemodynamic responses across multiple phases of active standing in individuals with and without long-COVID. By including not only blood pressure and heart rate, but also systemic vascular resistance, cardiac output, and even their coordination across time, this study provides a more integrated understanding of the dynamic mechanisms underlying orthostatic intolerance in this population. Unlike manual or

automated measurements, the use of continuous beat-by-beat measurement in this study also helps to identify transient and clinically meaningful drops in blood pressure, helping explain why individuals with long-COVID in this sample mentioned showing normal blood pressure during routine medical check-ups, despite experiencing orthostatic intolerance symptoms.

The findings also suggest that long-COVID may exist on a spectrum given that a portion of our control group met the criteria for OH. However, interpreting this observation is limited by the inability retrospectively confirm the absence of SARS-CoV-2 infection without relying on self-report. Also, this study did not include variables such as heart rate variability, baroreceptor sensitivity, or measures of muscle and brain oxygenation which might contribute to our understanding of compensatory mechanisms that prevented the emergence and report of OI symptoms in the control group. Therefore, future research should look into incorporating these measures to better understand the physiological mechanisms of asymptomatic OH.

Additionally, while normal orthostatic blood pressure drop (less than 20/10 mmHg) is usually accompanied by a compensatory increase in heart rate of approximately 10-20 bpm (Kavi et al., 2012; Raj, 2006), the observed pronounced BP drop (more than 20/10 mmHg) in the LC and NLC_{OH} groups accompanied by a HR response similar to the NLC_{NBPR} group with normal BP drop, suggests impairments in the baroreflex function, and could potentially predispose individuals with presyncope or fainting with prolonged standing, which could have occupational consequences for individuals in careers that require long hours of upright posture, such as healthcare workers, retail staff, etc. While no significant group differences were found across short-term phases in this study, examining baroreceptor sensitivity across these phases may provide information on the timing and adequacy of compensatory responses.

Finally, future studies should be conducted with a larger and more balanced sample size across groups, as doing so will strengthen the generalizability of findings and help clarify the physiological characteristics underlying both symptomatic long-COVID and asymptomatic individuals who nonetheless meet OH criteria.

Limitations

This study has limitations that should be considered. First, its single-centre, cross-sectional observational design limits casual interpretations. Although, to the best of our knowledge, this is the first study to assess a range of cardiovascular responses during both sit-to-stand and lie-to-stand tests in individuals with long-COVID, including short-term compensatory phases, the small sample size and uneven group distribution limits robustness of group comparisons. A *post-hoc* power analysis was conducted using G*Power (v3.1) for a one-way ANOVA with the three groups based on the observed effect size of SBP drop (G*Power effect size $f = 0.593$, derived from its partial eta squared $\eta^2 = 0.260$) and an alpha level of 0.05. The achieved power was 0.72, suggesting a 72% probability of detecting a true effect with the current sample size, which is below the conventional 80% (0.80) threshold (Cohen, 1992). An *post-hoc* power analysis (based on effect size f of SBP drop) also showed that 33 participants in total would have been required to achieve 80% power. Our sample fell short of this threshold, which potentially increases the likelihood of a type II error.

The majority of participants in this study were females, which may limit the generalizability of findings across sexes as sex differences were also not accounted for in our analysis. Given the known differences in autonomic function and cardiovascular responses between males and females, including differences in baroreflex sensitivity and vascular tone (Koenig & Thayer, 2016; Robert, 2023; Yeh et al., 2022), future studies should examine whether

these patterns are influenced by sex. Also, while the testing protocol included additional physiological measures such as heart rate variability, respiratory measures, and near infra-red spectroscopy for muscle and brain oxygenation, these variables were not analyzed in this thesis due to time limitations. However, these parameters will be explored in future work to complement and better understand the patterns observed in this study.

Conclusion

Based on the findings presented, long-COVID is associated with increased symptom reporting, signs of autonomic dysfunction and altered cardiovascular regulation during postural change. A more pronounced drop in BP, a blunted HR response and delayed CO-SVR matching for BP regulation following standing was observed in participants with long-COVID. The proportion of occurrence of orthostatic hypotension and orthostatic intolerance symptoms in the long-COVID group was 85% and 46%, respectively. Initial orthostatic hypotension was observed in 64% of long-COVID participants, while classical orthostatic hypotension was observed in 36%. Additionally, participants with long-COVID also rated significantly higher on 11 out of the 14 items of the DSQ-SF questionnaire, while only one met the Canadian Consensus Criteria for ME/CFS. However, the current study findings are limited by the small sample size; therefore, replication is warranted in a larger cohort to confirm these results.

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CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

This thesis aimed to investigate cardiovascular responses to an active standing orthostatic stress in individuals with long-COVID. Using a systematic review and a cross-sectional study, the goal was to understand the symptomatology and physiological profiles associated with orthostatic intolerance in this population.

The systematic review identified a gap in the literature, in that very few research studies have used continuous beat-by-beat measurements to assess physiological responses to an active standing test in individuals with long-COVID, despite the relevance of this method to identify changes during a postural transition that mimics activities of daily living. Only 3 studies met the inclusion criteria, largely due to the lack of a control group for comparison, limited use of continuous data, and the absence of a standardized definition of long-COVID. Physiological data showed a reduced heart rate variability and increased heart rate following standing, while blood pressure responses (specifically systolic) were variable across studies. Most studies focused primarily on SBP and HR, with limited reporting of other variables that could have contributed to a more integrated understanding of the patterns of response in this population.

To address some of these gaps, the cross-sectional study aimed to assess beat-by-beat cardiovascular responses during an active standing test, including looking at short-term compensatory mechanisms across four time specific phases. It included a control group for comparison, used the consensus definition of long-COVID from the National Academies of Science, Engineering and Medicine, and considered a few more cardiovascular variables, including SBP, DBP, MAP, SVR, HR, CO, SV and CO-SVR matching. The findings suggested that individuals with long-COVID reported significantly higher symptom burden on the DSQ-SF, similar to individuals with ME/CFS. A large proportion of the LC group also met the criteria

for orthostatic hypotension, with IOH being more prevalent than COH compared to the control group. Relating to cardiovascular responses, there was a more pronounced drop in SBP, DBP, MAP and SVR in the LC group, but HR responses were similar to those in the control group with normal BP drop, indicating a blunted HR response. Cardiac output to systemic vascular resistance matching patterns also differed, with a delayed recovery pattern in the LC group compared to the controls, indicating a less efficient compensatory mechanisms.

Together, both studies contribute to the existing literature on long-COVID, and while the achieved power in the cross-sectional study was slightly below ($0.72 - 72\%$) the recommended threshold of 0.8 (80%), the high frequency of occurrence of initial orthostatic hypotension in the long-COVID group, along with a delayed CO-SVR matching suggests potential clinical significance as the inability to fully maintain blood pressure and the presence of orthostatic hypotension (IOH and COH) and orthostatic intolerance related symptoms may lead to a risk for transient loss of consciousness. Although no participants in this study experienced syncope (probably due to the duration of 7 minutes), it raises the question and concern of whether such dysregulation could worsen over time.

The results also point towards the importance of individualized management strategies tailored to specific symptom presentations, as the identification of clustering of physiological responses (IOH or COH, blunted HR response, etc.) may help inform personalized approaches to symptom management. Some intervention suggestions, including the use of compression socks, performing calf raises before standing to help venous return, or encouraging slower postural transitions, may aid in minimizing the orthostatic intolerance symptoms.

In conclusion, more research is needed to validate the results of this study and explore long-term outcomes. Future work should continue the use of continuous beat-by-beat

monitoring, examine potential sex differences in physiological outcomes, and incorporate a broader set of cardiovascular, autonomic, and respiratory variables to better understand the dynamic physiological integration in this population.

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Appendix

Appendix A.

Definitions for COVID-19 severity according to the World Health Organization (WHO) and the National Institutes of Health (NIH). (COVID-19 Treatment Guidelines Panel, 2019; World Health Organization, 2021).

Asymptomatic	Test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test [NAAT] or an antigen test), but who have no symptoms.
Mild	May exhibit a variety of signs and symptoms (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell). They do not have shortness of breath, dyspnea on exertion, or abnormal imaging. Can be managed in an ambulatory setting or at home.
Moderate	Evidence of lower respiratory disease, during clinical assessment or imaging, signs of pneumonia (fever, cough, dyspnoea, fast breathing), but with SpO ₂ ≥ 94% on room air at sea level. Should be closely monitored.
Severe	Severe illness if they have SpO ₂ <94% on room air at sea level, PaO ₂ /FiO ₂ <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50% (signs of severe pneumonia, cough or difficulty in breathing). Oxygen therapy should be administered immediately. Hospitalized patients.
Critical	Respiratory failure, acute respiratory distress syndrome, septic shock, cardiac dysfunction, an exaggerated inflammatory response, and/or multiple organ dysfunction. May also experience hepatic, renal, central nervous system, or thrombotic diseases. Patient in the intensive care unit (ICU).

Appendix B.

STROBE Checklist

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix C.

Pretest Instructions

Please bring athletic clothing. It is strongly recommended to wear a loose-fitting t-shirt, loose-fitting shorts with pockets, and running shoes.

Please refrain from eating, drinking fluids of any kind, and smoking in the 2 hours leading up to your appointment time. Consumption of these substances will affect the test scores and therefore must be avoided. You will be able to drink water after the initial testing (about 10 - 15 minutes from your appointment start time).

Please refrain from drinking alcohol or performing strenuous exercise in the 24 hours leading up to your appointment time. These will also affect the test scores and therefore must be avoided.

On the day before your test, please maintain your regular dietary and sleeping patterns if possible and consume water throughout the course of the day. Since you will have to refrain from consuming anything in the 2 hours before the test, feeding and hydrating yourself adequately on the previous day is beneficial.

Appendix Supplemental 4.1.

Search Strategy

MEDLINE Search Strategy

Search string		Results
1	Middle Aged/ or Aged/ or Adult	20793566
2	Orthostatic intolerance/ or pure autonomic failure/	39447
3	Viral signs/ or blood pressure/ or heart rate/ or respiratory rate/	1251410
4	Posture/ or syncope/ or adult/ or syncope vasovagal/ or hypotension, orthostatic/ or postural orthostatic tachycardia syndrome/ or humans/ or blood pressure	57528966
5	Posture/ or active standing.mp. or Hypotension, orthostatic/	188996
6	Post-acute COVID-19 syndrome/ or long-covid.mp. or SARS-CoV-2/	663440
7	Covid-19/ or post-acute covid-19 syndrome/ or severe acute respiratory syndrome/	1114581
8	Long-haul covid.mp. or Post-acute COVID-19 syndrome/	15612
9	2 and 6	505
10	5 and 6	299
11	4 and 5 and 6 and 7	208

EMBASE Search Strategy

Search string		Results
1	((long-covid or post-covid* or long-haul covid or PASC) and active standing test).mp.	11
2	(((long-covid or post-covid* or long-haul covid or PASC) and active standing test) or orthostatic stress test or postural transition).mp.	142
3	((((long-covid or post-covid* or long-haul covid or PASC) and active standing test) or orthostatic stress test or postural transition) and hemodynamic responses) or orthostatic intolerance or orthostatic hypoten*).mp.	28689
4	Limit 4 to yr = “2020 – Current”	5276

CINAHL Search Strategy

Search string		Results
S9	S1 AND S6 (narrowed by language: English, and publication date: 2020 - 2025)	80
S8	S1 AND S6 (narrowed by age: all adult)	81
S7	S1 AND S6	438
S6	Post-acute sequelae of covid-19 OR PASC OR post-covid syndrome OR long haulers OR chronic COVID AND orthostatic stress test OR orthostatic challenge OR active standing OR tilt table test OR postural stress OR orthostatic intolerance AND cardiovascular response OR heart rate variability OR blood pressure regulation OR autonomic dysfunction OR orthostatic hypotension OR postural tachycardia syndrome	14, 545
S5	S1 and S2 and S4	1
S4	active stand test or orthostatic stress test and long-covid	17
S3	S1 AND S2	6
S2	orthostatic hypotension' or 'postural hypotension' or 'postural drop	2,673
S1	long covid or chronic covid-19 or post covid or long haul covid or post covid-19	7,114

PUBMED Search Strategy

Search string	Results
("long COVID"[Mesh] OR "post-acute sequelae of COVID-19" OR "PASC" OR "post-COVID syndrome" OR "long-haul COVID" OR "long haulers" OR "chronic COVID") AND ("orthostatic stress test" OR "orthostatic challenge" OR "active standing" OR "tilt table test" OR "postural stress" OR "orthostatic intolerance") AND ("cardiovascular response" OR "heart rate variability" OR "blood pressure regulation" OR "autonomic dysfunction" OR "orthostatic hypotension" OR "postural tachycardia syndrome")	14
(((((((("long-COVID"[MeSH Terms] OR "long-COVID"[All Fields] OR "long-COVID"[All Fields] OR "post-acute sequelae of COVID-19"[All Fields] OR "PASC"[All Fields] OR "post-COVID syndrome"[All Fields] OR "long-haul COVID"[All Fields] OR "long haulers"[All Fields] OR "chronic COVID"[All Fields]) OR ("COVID-19"[MeSH Terms] AND "sequelae"[All Fields]) AND (((("orthostatically"[All Fields] OR "orthostatics"[All Fields] OR "orthostatism"[All Fields] OR "standing position"[MeSH Terms] OR ("standing"[All Fields] AND "position"[All Fields]) OR "standing position"[All Fields] OR "orthostatic"[All Fields]) OR "orthostatic stress test"[All Fields] OR "active standing"[All Fields] OR "tilt table test"[All Fields] OR "postural stress"[All Fields] OR "head-up tilt"[All Fields] OR "head-up tilt test"[All Fields])) OR ("hypotension"[MeSH Terms] OR "hypotension"[All Fields] OR	53

	"orthostatic hypotension"[All Fields] OR "hypotensive"[All Fields]) OR "postural tachycardia syndrome"[All Fields] OR "POTS"[All Fields])) AND (((("cardiovascular response"[All Fields] OR "heart rate variability"[All Fields] OR "blood pressure regulation"[All Fields] OR "autonomic dysfunction"[All Fields]) OR "autonomic control"[All Fields] OR "autonomic regulation"[All Fields])))	
	head-up"[All Fields] AND "tilt"[All Fields] AND ("post acute covid 19 syndrome"[MeSH Terms] OR ("post acute"[All Fields] AND "covid 19"[All Fields] AND "syndrome"[All Fields]) OR "post acute covid 19 syndrome"[All Fields] OR ("long"[All Fields] AND "covid"[All Fields]) OR "long covid"[All Fields])	12
	((("blood pressure"[MeSH Terms] OR ("blood"[All Fields] AND "pressure"[All Fields]) OR "blood pressure"[All Fields] OR "blood pressure determination"[MeSH Terms] OR ("blood"[All Fields] AND "pressure"[All Fields] AND "determination"[All Fields]) OR "blood pressure determination"[All Fields] OR "arterial pressure"[MeSH Terms] OR ("arterial"[All Fields] AND "pressure"[All Fields]) OR "arterial pressure"[All Fields] OR ("heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields])) AND ("post acute covid 19 syndrome"[MeSH Terms] OR ("post-acute"[All Fields] AND "covid 19"[All Fields] AND "syndrome"[All Fields]) OR "post acute covid 19 syndrome"[All Fields] OR ("long"[All Fields] AND "covid"[All Fields]) OR "long covid"[All Fields])) OR "postcovid-19"[All Fields] OR	2

	"post cov*" [All Fields]) AND ("hypotension, orthostatic" [MeSH Terms] OR ("hypotension" [All Fields] AND "orthostatic" [All Fields]) OR "orthostatic hypotension" [All Fields] OR ("orthostatic" [All Fields] AND "hypotension" [All Fields])) AND ("post acute covid 19 syndrome" [MeSH Terms] OR ("post-acute" [All Fields] AND "covid 19" [All Fields] AND "syndrome" [All Fields]) OR "post acute covid 19 syndrome" [All Fields] OR ("long" [All Fields] AND "covid" [All Fields]) OR "long covid" [All Fields]) AND "long cov*" [All Fields] AND ("post-acute" [All Fields] AND "sequ*" [All Fields])	
	("haemodynamic" [All Fields] OR "hemodynamics" [MeSH Terms] OR "hemodynamics" [All Fields] OR "hemodynamic" [All Fields] OR "haemodynamical" [All Fields] OR "haemodynamically" [All Fields] OR "haemodynamics" [All Fields] OR "hemodynamical" [All Fields] OR "hemodynamically" [All Fields]) AND ("response" [All Fields] OR "responses" [All Fields] OR "responsive" [All Fields] OR "responsiveness" [All Fields] OR "responsivenesses" [All Fields] OR "responsives" [All Fields] OR "responsivities" [All Fields] OR "responsivity" [All Fields]) AND ("post acute covid 19 syndrome" [MeSH Terms] OR ("post acute" [All Fields] AND "covid 19" [All Fields] AND "syndrome" [All Fields]) OR "post acute covid 19 syndrome" [All Fields] OR ("long" [All Fields] AND "covid" [All Fields]) OR "long covid" [All Fields])	59

SCOPUS Search Strategy

	Search string	Results
	(ALL(long AND covid OR pasc OR long-haul AND covid OR chronic AND covid OR post-acute AND seque*) AND TITLE-ABS-KEY(active AND stand* OR orthostatic AND stress OR head AND up AND tilt OR hutt)) AND PUBYEAR > 2019 AND PUBYEAR < 2025	4

Appendix Supplemental 5.1

SAGER Guidelines

General principles

- Authors should use the terms *sex* and *gender* carefully in order to avoid confusing both terms.
- Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.
- Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction.

Recommendations per section of the article

Title and abstract	If only one sex is included in the study, or if the results of the study are to be applied to only one sex or gender, the title and the abstract should specify the sex of animals or any cells, tissues and other material derived from these and the sex and gender of human participants.
Introduction	Authors should report, where relevant, whether sex and/or gender differences may be expected.
Methods	Authors should report how sex and gender were taken into account in the design of the study, whether they ensured adequate representation of males and females, and justify the reasons for any exclusion of males or females.
Results	Where appropriate, data should be routinely presented disaggregated by sex and gender. Sex- and gender-based analyses should be reported regardless of positive or negative outcome. In clinical trials, data on withdrawals and dropouts should also be reported disaggregated by sex.
Discussion	The potential implications of sex and gender on the study results and analyses should be discussed. If a sex and gender analysis was not conducted, the rationale should be given. Authors should further discuss the implications of the lack of such analysis on the interpretation of the results.

Appendix Supplemental 5.2

Self-Reported Medication and Supplement Use by Participant

Participant ID	Medications and Supplements
P1	Sertraline hydrochloride (PTSD antidepressant), Esomeprazole Magnesium (Acid reflux)
P2	None
P3	None
P4	None
P5	None
P6	Birth control pills, Dextroamphetamine (ADHD), Supplements (vitamin D, lysine)
P7	Fluoxetine 20mg (anxiety, anti-depressant), Supplements (Iron, Vitamin D, B12), Birth control pills
P8	Supplements, Mirtazapine, CBD oil, Ivermectin
P9	Escitalopram (anti-anxiety); Paxlovid (a year ago)
P10	Concerta (ADHD)
P11	Supplements (Vitamin D, Vitamin C, Liver probiotic), Birth control pills
P12	Beta blockers (not specified)
P13	Symbicort (only in the fall and not daily), Rabeprazole (stomach ulcer, not daily), Naproxen
P14	None
P15	None

P16	None
P17	None
P18	Levebel levetiracetam
P19	None
P20	None
P21	Supplements (Vitamins D, K, C, B12, Omega 3, B complex, Ubiquinol, Cocuten, Nattokinase)
P22	None
P23	Isotopen (Anti-depressant), Ibuprofen (ADHD)
P24	None
P25	None
P26	None

This table summarizes self-reported medication and supplement use for each study participant.

Participants were asked to list all medications and supplements they were currently taking.

Reported items include prescription medications, over-the-counter medications, and dietary supplements. Some medications are listed with their use in parentheses for clarity (e.g., antidepressant, ADHD, etc.). ‘None’ indicates no medication or supplement use was reported.

Appendix Supplemental 5.3

Paffenbarger Physical Activity Questionnaire

PAFFENBARGER PHYSICAL ACTIVITY QUESTIONNAIRE		
We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in a typical week. Please answer each questions even if you do not consider yourself to be an active person. Please think about all the activities you do at work, as part of your house and yard work, to get from place to place, and in your space for recreation, exercise or sport.		
	Minutes/day OR Flights/day	Days/Week
Total number of blocks walked in a typical day. (12 blocks = 1 mile = 1.6 kilometers); 1 mile = 20 minutes. How many days/week?		
Total flight of stairs climbed UP in a typical day. (1 flight = 10 steps)		
Total minutes of LIGHT sport/recreation (cooking, gardening, walking etc) in a typical day. How many days/week?		
Total minutes of MODERATE sport/recreation (soccer, fencing, aerobics, home exercise etc) in a typical day OR muscle training. How many days/week?		
Total minutes of HEAVY sport/recreation (weight lifting, sowshoeing, ice hockey, boxing etc) in a typical day. How many days/week?		
This is the end of the questionnaire, thank you for participating.		
REPORT		
Kcal/week blocks walked		0
Kcal/week flight of stairs climbed		0
Light intensity kcal/week		0
Moderate intensity kcal/week		0
Heavy intensity kcal/week		0
Total		0

Appendix Supplemental 5.4

Consent Form

Participant Informed Consent Form

Long-COVID Study #1

Study Title: Differences in the autonomic, cardiovascular, and respiratory systems during postural transitions in individuals with and without long-COVID – An integrative approach

Student Principal Investigator:

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Student Advisor:

Dr. Rodrigo Villar, Assistant Professor

Faculty of Kinesiology and Recreation Management, University of Manitoba,

227 Active Living Centre, Winnipeg, Manitoba, R3T 2N2

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Email: rodrigo.villar@umanitoba.ca

Conflicts of Interest and Undue Influence:

None of the researchers have any conflicts of interest in this study/research.

You are being invited to participate in a research study. This consent form, a copy of which has been given to you, is only part of the process of informed consent. If you want more details about something mentioned here, or information not included here, feel free to ask any of the people

named above. Please take the time to read this document and any accompanying information carefully. It is very important that you understand:

- what is being asked of you,
- what the risks and benefits of participation are, and
- how the information you provide will be used and stored.

What is purpose of this research?

I am doing this research for my Master's thesis under the supervision of Dr. Rodrigo Villar. The overarching goal of this research is to determine how long-COVID impacts the brain, heart, vessels, and lungs regulation.

Study procedures

What will you be asked to do in the research? How much time will you commit?

As a participant, you will be asked to visit the research lab located in room #240 Applied Research Centre (ARC) at the University of Manitoba for one session. Prior to your arrival, you'll receive a document outlining pre-test instructions:

- Avoid alcohol and vigorous physical activity for 24 hours before the test.
- Refrain from consuming sugary or caffeinated beverages on the test day.
- Avoid large meals within two hours of your session.

Upon arrival, the principal investigator will confirm that you have adhered to these instructions.

If the instructions were not followed, the session will need to be rescheduled to ensure the validity of the data collected.

Pre-assessment

Measurements of baseline values and anthropometric values will be assessed.

- Height, weight, adipose tissue thickness, body fat percentage, waist and hip circumferences will be recorded.
- Resting heart rate, blood pressure, and oxygen saturation will also be measured.
 - o If your blood pressure exceeds **140/90 mmHg** or heart rate is above **100 bpm**, you will rest for 5 minutes, and a second reading will be taken.
 - o If your oxygen saturation is below **92%**, or elevated blood pressure and heart readings persist, the session will be rescheduled.

Postural transition protocol

You will perform two types of postural transitions (a) sit-to-stand and (b) lie-to-stand. Each transition will include:

- A) Sit to stand: Sit for 5 minutes and stand for up to 7 minutes.
- B) Lie to stand: Lie down for 10 minutes and stand for up to 7 minutes.

Each transition will be performed one time with a 10-minute break in-between.

Continuous measurement of physiological responses

Several physiological variables will be assessed during this protocol, including information about your central nervous system activity (brain, e.g., sympathetic and parasympathetic), cardiovascular (e.g., heart rate and blood pressure), and respiratory (e.g., oxygen consumption and carbon dioxide output) systems responses. This visit will take approximately 3 hours.

Measurement devices

During the postural transition protocol, your cardiovascular responses, including blood pressure, heart rate, and cardiac output, will be continuously measured beat-by-beat using a device called Finometer Pro. Your respiratory responses, including oxygen consumption, carbon dioxide production, ventilation, respiratory frequency, and respiratory exchange ratio, will be measured

breath-by-breath using a metabolic cart. Your total hemoglobin, oxyhemoglobin (oxygen attached to the hemoglobin), deoxyhemoglobin (hemoglobin with no oxygen attached), and blood volume will be measured using near-infrared spectroscopy (NIRS) device.

Cardiovascular measurement devices

Your blood pressure will be measured non-invasively by a small cuff wrapped around your middle finger on either right or left hand with your palm facing upwards. Once the cuff is accurately placed on your finger, the cuff will be secured into place using Velcro fasteners. The finger cuff will apply light pressure around your finger to measure blood pressure for every heartbeat. This should not cause any discomfort to you other than a different perception of your finger. A heating pad may be placed over your hand to keep it warm throughout the test if it is necessary. Your cardiac output will be determined by the model flow algorithm used in the Finometer device.

Your heart rate will be monitored by a three-lead electrocardiogram (ECG). Three disposable, single-use electrodes will be placed just below your right (electrode 1) and your left (electrode 2) collar bone (clavicle), and one on the lower left side of your ribs (electrode 3). To improve signal quality, first, a cleaning procedure will be performed in the area where the electrocardiogram electrodes will be placed, as follows: if you have hair in the area, your skin will be shaved with a hair clipper, cleaned with alcohol swabs, and gently rubbed with fine-grain emery paper or gauze pad to remove the superficial skin layer. The researcher will then place the electrodes in the areas previously described. There is a minimal chance that you might develop a minimal skin rash from the adhesive on the electrodes, but there is no way of knowing this before testing. If a rash develops, you will be instructed to avoid scratching, keep the area clean using an alcohol swab and moisturize the area with body lotion. The rash will go away within a day or so.

Respiratory measurement devices

A metabolic cart will measure your respiratory responses. For this purpose, you will be fitted with a mask that will be attached to a metabolic cart that will monitor and record your ventilatory variables (e.g., oxygen consumption, carbon dioxide output).

Autonomic measurement devices

Your skeletal muscle oxygenation (total hemoglobin, oxyhemoglobin, deoxyhemoglobin, and blood volume estimation) will be measured by Near-Infrared Spectroscopy (NIRS). The NIRS device uses small non-invasive probes that will be placed on your leg (around your calf and thigh muscles). The NIRS probe will be held on your skin by a support system designed specifically for this purpose (a flexible elastic band). The research assistant will remove the NIRS device from your skin at the end of data collection.

All of the measurements mentioned use non-invasive equipment and will be done by a trained researcher. After data collection, all equipment will be sanitized with alcohol or other bactericidal solution. You will be asked if you have any known sensitivity to this sanitizing agent and, if so, advised that you should not participate in the study. You will be allowed to remove any of the devices at any time if you feel uncomfortable or if you are not feeling well, and the test will be terminated immediately.

What should I wear for the experimental visit?

Comfortable exercise clothing, similar to what you would wear to the gym, should be worn or brought to the testing sessions. Shorts are required and should be worn with running shoes to ensure the NIRS device can be placed in the correct location on your leg. You must wear a T-Shirt or tank top to allow the researcher to place the ECG electrodes.

Study Risks

Inclusion in this study requires participants to have no pre-existing chronic health conditions affecting the cardiovascular, autonomic, and respiratory system. This criterion is important to ensure both participant safety and the validity of the data, as such conditions could influence physiologic responses to the postural transition test and invalidate data interpretation.

While the risks associated with this protocol are minimal, there is a slight possibility of experiencing light-headedness, dizziness, or fainting. These events are rare, but your feedback, along with continuous beat-by-beat blood pressure and heart rate monitoring, will help us anticipate and address any such occurrences immediately. In the case of severe symptoms, the test will be stopped immediately.

The risks involved in performing postural transitions are comparable to everyday activities, such as getting out of bed in the morning or standing up from a chair. However, in the unlikely event of more severe adverse events (e.g., fainting or cardiac events), emergency procedures and basic medical equipment will be in place to ensure you have immediate care until paramedical or medical assistance arrives. There is also an emergency button on the same floor level as our research laboratory that directly contacts the building's emergency services.

Study Benefits

You will have access to information related to your health (e.g., body weight, body fat, and blood pressure) and be educated about how important and intertwined your heart, lungs, and brain responses are to perform simple tasks such as getting out of your bed in the morning. The indirect benefits would include researchers obtaining a better understanding of how the autonomic, cardiovascular, respiratory, and musculoskeletal systems respond to orthostatic stresses (postural transitions), which is crucial for maintaining homeostasis, functional mobility, and survival.

Compensation

Parking expenses will be covered once you consent to participate, and a test date has been scheduled. A parking spot will be reserved for you in advance. If you decide to withdraw from the study after arriving on campus, you will still be reimbursed for any parking expenses incurred during your visit to the lab.

After the study is done, will I find out the results?

If you desire, we will send a brief summary of the project results shortly after completing the data analysis. You will be asked how you would like to receive this information at your first experimental visit (i.e. via email or in person) and will be told when you can expect to receive it (estimated end of August 2025). Feedback will be provided to you by the principal investigator or research assistant involved in this study by the mode you chose. You may also choose not to receive feedback about the study.

Use and Storage of Information

All identifiable data (e.g., name, email address) will be retained until data analysis begins in March 2025. After this point, it will be securely destroyed, making sure that the data can no longer be linked back to you. When we share the results of this study, we will present combined data so that your individual responses remain confidential. If any specific mentions are necessary, they will be described using general terms such as "one female participant" or "one male participant", with male or female referring to biological sex.

To manage your data, a unique code will be assigned that cannot be linked specifically to you. This code will help us organize the physiological data we collect, which will remain non-identifiable. This non-identifiable physiological data will also be kept indefinitely as we build a

database of physiological responses to aid in conducting more comprehensive secondary analysis on long-COVID.

At the end of this document, you can indicate whether you would like to receive results from this study (expected date – August 2025). If you wish to receive the results, your name and contact information will be saved in a password-protected file on Dr. Rodrigo Villar's University of Manitoba SharePoint account, accessible only to him. If you do not want to receive this, you are not required to provide any contact information. Consent forms will be stored in a locked filing cabinet (Room 227, Active Living Centre, FCRM, University of Manitoba) for five years and securely destroyed afterward (December 2029). You can also indicate if you'd like to be contacted for future studies by providing your contact information in the space provided below. As this information will be on the consent form, it will also only be retained for 5 years (December 2029), after which it will be securely destroyed.

All information you provide as part of this study is confidential, meaning we will take every measure to keep it secure. However, absolute confidentiality cannot be guaranteed. Personal information will only be shared if required by law or court order (e.g., in cases of suspected child or elder abuse).

Your physiological data may also be shared outside the University of Manitoba with other researchers, organizations, or made publicly available for further analysis, or as required by an academic journal. In such cases, no identifying information (e.g., name) will be included.

Dissemination

The study results will be shared through 1) the publication of a master's thesis; 2) journal publications and presentations in relevant academic fields; and 3) a summarized report of the study results to be sent to interested participants.

Withdrawal from the study

Your participation in this research study is voluntary. You can choose to do only the activities and/or answer only the questions that you are comfortable with. You may withdraw from the study for any reason. You do not have to explain why. You will not be penalized in any way. Should you withdraw partway through the study, all of your information will be destroyed unless you have consented to allowing us to keep it. You may withdraw from the study until **March 2025**. After this date, we will start our analysis so it may not be possible to withdraw your information as it will not be linked to your name. To withdraw, please contact Faith Olarinde or Dr. Rodrigo Villar at the phone number or email above.

Questions or Concerns

Designated University of Manitoba personnel may check that this study is being done safely and properly. To do this, they may visit the study site or review the research records. We will tell you if someone outside the research team will be there while you are participating. If this makes you uncomfortable, please tell a member of the research team, who will ask the personnel to return at another time.

This study has been reviewed and approved by a Research Ethics Board at the University of Manitoba. However, this does not mean that participation is risk-free. If you have questions about your rights as a research participant, you may contact the Office of Human Research Ethics at humanethics@umanitoba.ca or (204) 474-7122.

If you have any questions, concerns, or complaints about this study, you may contact any members of the research team listed on the first page or the Office of Human Research Ethics.

Consent

By signing this document, I agree that:

- I have read the above information or had it read to me.
- I have had the opportunity to ask and have answered all of my questions.
- I understand what is being asked of me.
- I will be taking part in a research study.
- I may freely stop or leave the research study activities at any time.
- My information may be shared outside the University of Manitoba.
- I do not waive my legal rights by participating in the study.

Notice Regarding Collection, Use, and Disclosure of Personal Information

Your personal information is being collected under the authority of The University of Manitoba Act. The University of Manitoba is committed to preserving your right to privacy. The information you provide will be used by the University to support our research. Your personal information will not be used or disclosed for other purposes, unless permitted by The Freedom of Information and Protection of Privacy Act or The Personal Health Information Act. If you have any questions about the collection of personal information: Ph: 204-474-9462 or Email: fippa@umanitoba.ca

I agree to participate in this study.	☐ YES ☐ NO
Please provide an email address below if you would like to be sent a summary of the study results and/or my individual results (Expected date: August 2025). Email address: _____	

I agree to be contacted by a member of this research team for future studies via the contact information provided above.

☐ YES ☐ NO

By agreeing to be contacted, you are not required to participate in any future phases. If you wish to withdraw your name from this list, please contact Dr. Villar at rodrigo.villar@umanitoba.ca.

Email address:

Name of Participant

Participant's Signature

Date